NATURALLY-OCCURRING AND EXPERIMENTALLY-INDUCED CHANGES IN ESTROGEN AND CONSEQUENT DIETARY SELF-SELCTION

BY

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This doctoral dissertation is dedicated to my wife, Sue, who withstood the trials and tribultions of living with and supporting a graduate student for more years than either of us wish to remember.

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NATURALLY-OCCURRING AND EXPERIMENTALLY-INDUCED CHANGES IN ESTROGEN AND CONSEQUENT DIETARY SELF-SELECTION

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Rhythmic changes in food intake and body weight accompany naturally-occurring fluctuations of the ovarian hormones during estrous cycles of rats coincident with estrogenic stimulation. The present experiments examined dietary-selection patterns of female intact and ovariectomized rats with changing estrogenic stimulation. An assessment of the effects of estrogenic stimulation on diet selection based on caloric density was examined in groups of ovariectomized rats presented with pairs of diets to representing one of the three basic macronutrients by varying the percentage of one of the macronutrients in each pair. Thus, caloric density was consequently varied. Following ovariectomy, an almost exclusive preference for the more calorically dense diet occurred, regardless of macronutrient pair, an effect suppressed by estradiol benzoate administration. A macronutrient specific effect was suggested by the greater suppression of the high

carbohydrate diet. Intact cycling female rats presented with an isocaloric diet triplet of fat, carbohydrate, and protein did not vary total caloric or water intake in addition to body weight across the estrous cycle. However, diet selection did vary with fat intake increasing and carbohydrate and, to a lesser extent, protein intake decreasing. Conversely, the opposite dietary selection pattern occurred during diestrus. Following ovariectomy, these rats progressively increased their intakes of carbohydrate and protein with a concurrent decrease in fat consumption. Estrogenic stimulation partially reversed this diet selection profile. In general, further confirmation of the above results using the diet triplet paradigm occurred with all possible combinations of these diets presented as pairs, both with naturally-occurring and experimentally-produced estrogenic stimulation. To summarize, fat intake is inversely related to estrogenic stimulation while that of carbohydrate and protein are directly related to such stimulation. These data demonstrate that estrogenic stimulation does not necessarily suppress caloric or water intake, and body weight. These results are also consistent with the past 40 years of research using standard laboratory chow, a diet composed of over 50% carbohydrate.

CHAPTER I

GENERAL INTRODUCTION

Ovarian hormones have been shown to affect a variety of behaviors in rats. Of these behaviors, over forty years of research has focused on the effects of these hormones on reproductive behaviors and energy regulation (i.e., food intake and energy expended as locomotor activity) (for review see Phoenix, Goy, & Young, 1967; Wade, 1972, 1976; Wade & Gray, 1979). In terms of energy regulation, it has been well established that fluctuations in the circulating levels of the ovarian hormones affect food intake, body weight, carcass composition, and energy expended as locomotor activity (vide infra).

Although there are two classes of ovarian hormones, the estrogens and progestins, it appears that it is the estrogens, particularly 17-beta-estradiol (E2), that are responsible for the changes in energy regulation (vide infra). In general, circulating E2 titers are negatively correlated with food intake, body weight, and carcass fat, and positively correlated with locomotor activity (vide infra). Food intake (e.g., ter Haar, 1972), voluntary exercise (e.g., Simpson & May, 1973), and taste responsiveness (e.g., Zucker, 1969) all vary with the estrous cycle as determined by changes in the vaginal cell sample. During the first two stages of the estrous cycle (i.e., proestrus and estrus, respectively) circulating titers of E2 are highest and food

intake is suppressed. During the last two stages of the estrous cycle (i.e., metestrus and diestrus, respectively) food intake is increased and E2 titers are lowest (ter Haar, 1972).

Experimental support for the modulatory role of E2 on food intake and the antagonistic role of progesterone, the primary ovarian progestin, comes from the use of the gonadectomy-replacement paradigm. After removal of the ovaries, the primary source of the estrogens and progestins, food intake and body weight increase for approximately three to four weeks (e.g., Kakolewski, Cox, & Valenstein, 1968; Stotsenburg, 1913) and energy expended as locomotor activity decreases (e.g., Wang, 1923). Restoration of pre-ovariectomy food intake, exercise and body weight can be readily accomplished by daily "physiological" doses of estradiol benzoate (EB) (e.g., Stern & Murphy, 1972; Tarttelin & Gorski, 1973; Wade, 1975; Zucker, 1969, 1972). Progesterone replacement therapy in ovariectomized rats does not affect food intake, locomotor activity, or body weight (for review see Wade, 1972, 1976; Wade & Gray, 1979). On the other hand, progesterone administration to ovariectomized-EB treated rats will diminish or block the effects of EB on food intake and body weight (e.g., Roberts, Kenney, & Mook, 1972; Rodier, 1971; Ross & Zucker, 1974; Wade, 1975; Zucker, 1969). These results and others have led a number of investigators to postulate an antagonistic role of progesterone for E2 in regard to food intake (Wade, 1972, 1976; Wade & Zucker, 1969; Zucker, 1972) and reproductive behavior (Morin, 1977) and a primary role for E2.

Although the effects of E2 on body weight, carcass composition, and food intake are well established, the physiological basis of these

effects have not been unified into an overall scheme that has been experimentally verified. For example, Wade (1972) suggested that E2 may work on separate neural circuitries that decrease food intake and increase locomotor activity via the lowering of a "hypothalamic lipostat." While the utility of concepts such as "lipostat" or "set point" are beyond the scope of this discussion (see Friedman & Stricker, 1976, for a treatment of this topic), it should be apparent that the act of naming a controller for a set of behaviors does not increase our understanding of the stimulus and response relations responsible for the behavior in question nor is it heuristically valuable.

Recently, however, a metabolic hypothesis has been advanced by Wade and Gray (1979) that is testable. Specifically, Wade and Gray (1979) proposed that the ovarian hormones alter fat deposition by varying the rate of storage of circulating triglycerides, a utilizable metabolic fuel, in adipose tissue. Increases in circulating metabolic fuels should decrease feeding while decreases should promote feeding (Friedman & Stricker, 1976). Using the gonadectomy-replacement paradigm, a number of effects on adipose tissue have been shown for E2 and progesterone. As stated earlier, ovariectomy (OVX) results in an increase in body weight primarily as body fat (e.g., Leshner & Collier, 1973). An important contributing factor in this and other forms of obesity may be the regulation of adipose tissue lipoprotein lipase (LPL) activity. This enzyme regulates the uptake of triglycerides into adipocytes (Garfinkel, Baker, & Schotz, 1967; Robinson & Wing, 1972) and has been found to be increased after OVX

(Wade & Gray, 1979; Hamosh & Hamosh, 1975) and in the development of obesity in the genetically obese Zucker fatty rat (Gruen, Hietanen, & Greenwood, 1979). Decreasing levels of circulating metabolic fuels caused by changes in LPL activity could result in food intake increases (for review see Friedman & Stricker, 1976). Administration of "physiological" doses of E2 decrease LPL activity (Hamosh & Hamosh, 1975), body fat (Roy & Wade, 1977), and, as stated earlier, food intake.

Further evidence for the feasability of the metabolic hypothesis of Wade and Gray (1979) are the findings that adipose tissue has cytoplasmic E2 receptors (Wade & Gray, 1978), and E2 administration induces the formation of adipose tissue progestin receptors (Gray & Wade, 1979) which could mediate the action of E2 and progesterone on adipose tissue LPL activity. In addition, E2 could affect enzyme activity by altering hepatic production of lipoprotein apoproteins which serve as cofactors for LPL activity (Kim & Kalkhoff, 1978). In summary, Wade and Gray (1979) propose that it is the ovarian hormonal modulation of adipose tissue uptake of triglycerides by increases and decreases in LPL activity, that alters circulating metabolic fuels which in turn control the intake of nutrients.

Indirect support for this metabolic hypothesis comes from the data of Bernardis, Bellinger, Goldman, and MacKenzie (1981) who found no change in lipogenesis from glucose or incorporation of glucose into glycogen in ovariectomized rats (in contrast see Matute & Kalkoff, 1973). Thus, the utilization of glucose, normally the major energy fuel, does not appear to be affected by OVX leaving the major energy

reserve, adipose tissue, as a possible site for ovarian hormone modulation of circulating metabolic fuels.

While the metabolic hypothesis of Wade and Gray (1979) is intuitively appealing, a direct test of this hypothesis casts some doubt on its validity. Ramirez (1980) predicted that, if Wade and Gray (1979) were correct, an increase in circulating triglycerides should precede or coincide with an E2-induced decrease in food intake and body weight. Ramirez (1980) found, however, that after four days of E2 replacement therapy in ovariectomized rats, food and body weight decreased but plasma triglycerides or free glycerol were not affected. A modification of the Wade and Gray (1979) metabolic hypothesis that focusses on free fatty acids as the major circulatory metabolic fuel derived from adipose tissue, in contrast to triglycerides, salvages this hypothesis. Ramirez (1980) did find a concomitant increase in circulating free fatty acids as food intake and body weight decreased. Thus, it may be circulating free fatty acids that are modulated by the ovarian hormones and consequently affect food intake. Further experimentation awaits possible confirmation of this revised metabolic hypothesis.

Alternatively, the metabolic hypothesis of Wade and Gray (1979) may not be correct in any form. Another obvious peripheral metabolic factor may be changes in insulin secretion produced by the ovarian hormones. It has long been known that increased circulating insulin can increase food intake while decreased insulin is normally correlated with low levels of food intake. However, in ovariectomized rats with

streptozotocin-induced diabetes, E2 is still effective in suppressing food intake suggesting the anorexic effects of E2 are not mediated by decreased insulin secretion (Dudly, Gentry, Silverman,& Wade, 1979). Additional support for the lack of a significant role of insulin in the ovarian hormonal modulation of food intake comes from the observation that glucose tolerance and plasma insulin levels are greatest during estrus, a time when food intake is decreased, not increased, and that ovariectomized rats given E2 show improved glucose tolerance and increased plasma insulin, yet decrease their food intake (Bailey & Matty, 1972).

It may be, however, that the ovarian hormones activate central nervous system estrogen and progestin receptors. Both progestin (e.g., Sar & Stumpf, 1973) and estrogen (e.g., Luine & McEwen, 1978) receptors have been found in rat brain. In fact, central administration of E2 in ovariectomized rats has been repeatedly shown to result in decreased food intake and body weight (e.g., Wade & Zucker, 1970). Furthermore, anatomical specificity has been shown for the central administration of E2 and subsequent decrease in food intake and body weight. Wade and Zucker (1970) have reported that the ventral medial hypothalamus (VMH) appears to be the most effective site for suppression of food intake and body weight, with other diencephalic sites much less or not effective. In addition, central administration of the "antiestrogen" MER-25 into the VMH in rats (Roy, Maass, & Wade, 1977) shows full estrogenic activity with respect to ingestive behavior as it does when administered peripherally (Roy & Wade, 1975).

The primacy of the VMH area as a site for E2's reduction in food intake came into question when rats with VMH lesions also decreased their food intake after systemic E2 administration (Beatty, O'Briant, & Vilberg, 1975). This work suggested that E2 may also be acting peripherally and/or centrally at a different site. Recently, Donohoe and Stevens (1981) found fully responsive amygdaloid sites with respect to the suppression of food intake, and also found that ovariectomized rats with VMH lesions reduced their food intake after amygdaloid E2 implants. This study suggests that both the amygdala and the VMH appear to be target tissues for the anorexic effect of E2.

The importance of estrogenic stimulation of the central nervous system and a subsequent anorexia has been shown by Nunez, Gray, and Wade (1980). E2 was implanted into the VMH area and adipose tissue LPL and cytoplasmic progestin receptor formation measured in addition to food intake and body weight, the former two measures being estrogen-sensitive endpoints. Within 24 hr after EB implantation, food intake and body weight were significantly suppressed, but there was not a change in adipose tissue LPL activity nor induction of progestin receptors. These results suggest that central estrogenic stimulation alone is sufficient to produce estrogenic effects on food intake. Of course, it should be noted that central and peripheral estrogen-stimulated effects are not mutually exclusive.

Perhaps the reduction in body weight and food intake are a result of an increase in energy expenditure as locomotor activity or thermogenesis. As stated earlier, it has been repeatedly demonstrated that

when circulating E2 levels are high, locomotor activity increases. Decreases in body weight and food intake also seen during these conditions may be due to the utilization of calories during exercise and response competition, respectively. However, three findings seem to question such an interpretation. First, separate neurologies have been implicated mediating the E2 stimulation of locomotor activity and the suppression of feeding. Specifically, Wade and Zucker (1970) found diencephalic sites that, when stimulated with EB, would result in either locomotor or feeding effects but not both. Second, the antiestrogen MER-25 that displays full estrogenic activity in terms of decreasing food intake and body weight (whether administered peripherally or centrally), does not affect locomotor activity (Roy & Wade, 1975). Third, if physical restraint is utilized, E2 replacement therapy in OVX rats results in an increase in heat production that may in itself be sufficient to produce the reductions in body weight and food intake (Laudenslager, Wilkinson, Carlisle, & Hammel, 1980). Therefore, it appears that increased energy expenditure in and of itself is insufficient to account for the estrogenic reductions in body weight and ingestive behavior.

In conclusion, it is currently not known what the physiological underpinnings are for either OVX-induced obesity or its reversal by E2 replacement therapy. Although the metabolic hypothesis of Wade & Gray (1979) appears appealing, estrogenic central nervous system stimulation alone appears sufficient to account for the effects of E2 on ingestive behavior (Nunez et al., 1980).

In the search for the physiological basis of the effects of the ovarian hormones on ingestive behavior nearly forty years of research has not been successful in any positive respect except for the elimination of possible factors. This elimination process could and will continue but perhaps a more fruitful approach may be to become more sophisticated in the behavioral paradigm employed in an attempt to allow the animal's behavior to direct future physiological studies. One such paradigm that has been proven successful in past work is the use of the dietary self-selection or cafeteria style paradigm as pioneered by Curt Richter (Richter, 1943).

A large literature exists on the ability of animals to select their dietary intake from a number of sources resulting in improved health as measured by both physiological and behavioral measures. A clear example of the utility of the dietary self-selection in leading to further work investigating physiology-behavior relations is the enhanced intake of sodium by adrenalectomized rats. In an extensive series of studies on the effect of adrenalectomy on dietary self-selection, Richter (1941) noticed both an increase in urinary output and an increase in the intake of sodium salts. This led to an investigation of the adrenal for a factor relating to sodium appetite and retention resulting ultimately in the discovery of the adrenocortical hormone, aldosterone. Thus, the critical use of the dietary self-selection procedure led to a focus on a specific nutrient and the role of blood-borne factor that affects the fate of this nutrient.

Similarly, the use of the dietary self-selection procedure may elucidate the ovarian hormonal control over macronutrient self-selection and these data may lead to future studies of protein, carbohydrate (CHO), and/or fat metabolism. Prior to the present experiment, six studies have attempted to investigate the role of the ovarian hormones on dietary self-selection and have yielded conflicting results for reasons to be discussed below.

Curt Richter, who realized the potential power of this behavioral paradigm, was the first investigator to examine the changes in diet selection in intact, cycling female rats (Richter, Holt, & Barelare, 1937, 1938) but did not relate the changes he observed to the stages of the estrous cycle. Using the same purified diets as Richter and his associates (Richter et al., 1937, 1938), Geiselman, Martin, VanderWeele and Novin (1981) obtained vaginal smears from their rats in order to make comparisons between estrous cycle stage and macronutrient selection. These researchers report an increase in CHO intake during estrus and concurrent decrease in the intake of fat. Conversely, during diestrus, an increase in the intake of fat was observed while that of CHO decreased. Protein intake reliably decreased during estrus but was not statistically significant. There was no change in caloric intake across the estrous cycle. In rats that were ovariectomized on day five postnatally and tested when adults, fat intake increased and CHO intake decreased. It was concluded that the dietary self-selection pattern of their ovariectomized rats reflected a similar condition to that of diestrus.

The primary factor compromising the results of Geiselman et al. (1981) is their failure to equate the caloric density of their diets. If one considers that a gram of fat contains approximately 9.0 kcal while that of CHO and protein contain about 4 kcal per gram, then it is clear that the olive oil source of fat is 2.5 times more caloric-ally dense than that of their CHO (sucrose) or protein (casein) source. This lack of equating caloric density has been shown to be especially important for fat diets in that normally, the feeding of high fat diets results in increased caloric intake, body weight, and fat dis-position (for review see Kanarek & Hirsch, 1977) but does not occur if the caloric density is reduced (Eisenstein, Strack, & Steiner, 1974; Zarazoga & Felber, 1970). Therefore, the effects of the ovar-ian hormones or their removal may be to alter diet selection based on a more general factor such as caloric density rather than some factor as specific as macronutrient content. That is, it seems nec-essary to equate the caloric density of the experimental diets in assessing the possible role of the ovarian hormones in macronutrient self-selection and therefore, the conclusions of the Geiselman et al. (1981) study appear tenuous. It should also be noted that between group comparisons were made between rats that had experienced the organizational aspects of hormonal control of behavior (for review, see MacLusky & Naftolin, 1981; McEwen, 1980) and neonatally, ovari-ectomized rats tested as adults but not experiencing these early hormonal influences.

Kanarek and Beck (1980) have recently reported that OVX does not alter macronutrient self-selection however this study also did not equate caloric density, did not state whether the rats used were exhibiting estrous cycles prior to surgery and compared post OVX self-selection data with pre-OVX data that had been collapsed across a number of days, apparently assuming that dietary self-selection patterns do not change with the changing ovarian hormonal status of intact, cycling female rats, if indeed their rats were cycling.

Leshner and Collier (1973) examined dietary self-selection patterns in intact and ovariectomized rats offered isocaloric diet pairs varying in protein or CHO content. These workers found protein intake to be increased after OVX but these results are difficult to interpret because a dietary fat source was not separately offered.

Lastly, Wurtman and Baum (1980), also using isocaloric diets lacking a separate fat source and achieving caloric equality between diet pairs by varying the content of a different macronutrient, report an estrogenic suppression of CHO but not protein when exogenous or endogenous E2 plasma titers are elevated. It should be clear that these results are compromised by both the lack of a separate fat source and the potential comfound in varying macronutrient content within a diet pair to obtain caloric equality.

The present set of experiments was designed to test and/or overcome some of the potential methodological and conceptual problems with the dietary self-selection studies reported to date concerning the role of the ovarian hormones in food intake. First, a more general

question was addressed instead of the more narrow question of estrogenic control of macronutrient intake. Rather, does estrogenic stimulation or its absence, affect the selection of diet based on caloric density? Secondly, is there an effect of the naturally-occurring fluctuations in ovarian hormonal secretion during the estrous cycle on dietary self-selection? Thirdly, is the effect on naturally-occurring modification of diet selection seen across the estrous cycle primarily due to E2? Fourth, does removal of the ovaries and the developing obesity alter dietary self-selection immediately, similar to the changes seen after adrenalectomy and salt intake (e.g., Richter, 1943) or do the alterations develop more gradually suggesting a "learned" component to the dietary changes observed? Fifth, can exogenously administered ovarian hormones, specifically E2, produce a similar dietary selection intake pattern to that during naturally-occurring estrogenic stimulation (i.e., estrus)? Lastly, does changing the dietary choices available change the effect of naturally-occurring or experimentally-induced ovarian hormonal control over macronutrient selection?

CHAPTER II

GENERAL METHOD

Subjects

Adult, female hooded rats bred at the University of Florida from stock purchased by a commercial breeder (Charles River Co., Wilmington, MA), weighing 239-340 gm served as subjects for all experiments. Three weeks prior to the beginning of the experiments each animal was individually housed in Wahmann LC-75 wire bottom cages adapted with either two or three aluminum strips secured to the front of the cage and fashioned into a circular holder for glass jars (6 oz.) that contained powdered Purina Rodent Chow 5001. A ¼" mesh screen rested atop the food to minimize spillage. Tap water was available ad libitum. During the three week adaption period, only one of the two or three glass jars was filled with the powdered chow and its position changed daily to minimize the development of position preference (e.g., Bartness & Alferink, 1979). Body weight and food and water intake were measured daily in the middle of the light portion of the 12:12 hour light/dark cycle. Room temperature was maintained at 22 ± 3° C. Food intake was corrected for spillage throughout the experiments.

Surgery

For those animals that underwent OVX, the rats were anesthetized with ether and their ovaries removed through bilateral incisions made on the flank, posterior to the rib cage.

Estrous Stage Determination

In the intact animals, vaginal smears were taken daily just prior to the beginning of the dark cycle using tap water and a wire loop. The smears were stained with toludine blue and examined under a light microscope. Although a standard nomenclature has not been established for estrous cycle stages (Wade, 1976), vaginal estrous cycle stage was determined according to the fluctuations of leucocytes, nucleated epithelial and cornified epithelial cells. The four stages of the estrous cycle were determined by the criteria of Gorbman and Bern (1962). Briefly, these stages were distinguished with proestrus defined as dominance of nucleated epithelial cells, estrus as dominance of cornified epithelial cells, metestrus as the presence of leucocytes and cornified cells and diestrus as the presence of leucocytes and nucleated epithelial cells (see Dewsbury, Estep, and Lanier, 1977, for further details). It should be noted that vaginal smearing does not affect food or water intake, or body weight (Young, Nance, & Gorski, 1978) and is highly correlated with ovarian hormonal levels (ter Haar, 1972).

Estrogen Replacement Therapy

When appropriate, ovariectomized rats received single subcutaneous injections of estradial benzoate (EB) (Schering Corp., Bloomfield, NJ) (3 or 9 ug/kg) or the sunflower oil vehicle in a volume of .1 ml/ 100 kg body weight. These doses were chosen to be above and below a dose of EB (6 ug) that has previously been shown to significantly decrease food intake and body weight (Nance & Gorski, 1975).

Ovariectomy Verification and Carcass Analysis

At the termination of experiments employing ovariectomized rats, the animals were killed by an overdose of sodium pentobarbital and a laperectomy performed to verify the absence of ovarian tissue. In addition, the rats were weighed and had their nose-anal lengths measured.

Statistical Analysis

In general, absolute and relative measures were analyzed using analysis of variance (ANOVA) for repeated measures. Simple main effects and/or main effects were followed by Duncan's New Multiple Range (DNMR) tests on statistically significant interactions that appeared to be logically significant (Lindman, 1974). A priori t-tests were performed on comparisons between means when appropriate, with the alpha level of protection distributed across the small sets of comparisons (Kirk, 1968).

CHAPTER III

EXPERIMENT 1:
ROLE OF CALORIC DENSITY IN OVARIECTOMY-INDUCED OBESITY

Caloric density has been shown to affect food intake in a number of situations including intact and hypothalamic lesion-induced obese rats (Levison, Frommer, & Vance, 1973). It has also been reported that female rats are better able to compensate for caloric dilution by increasing their food intake than male rats (Nance & Gorski, 1977). Moreover, this apparent enhanced ability to regulate total caloric intake by female rats is eliminated by OVX and restored by EB administration (Nance & Gorski, 1977). While differences in food intake are difficult to assess in rats of different sexes and most likely different body weights, this work suggests an estrogenic component to dietary self-selection based on caloric density. The purpose of Experiment 1 was to determine the role of caloric density in OVX-induced obesity.

Method

Subjects

Thirty-six adult female rats were divided into three groups of twelve equated for body weight ($\bar{X}$ ± SEM: 278.8 ± 9.8, 284.1 ± 9.5, 278.7 ± 9.4 g). All animals received bilateral ovariectomies as described above.

Procedure

Two weeks postsurgery the rats had their maintenance diet of powdered chow (Purina Rodent Chow 5001) changed to a maintenance diet of 67% powdered chow, to which was added either 33% protein (vitamin test free casein, Teklad Test Diets, Madison, WI; 3.74 kcal/gm) or 16.5% protein (casein) plus 16.5% nonnutritive cellulose (non-nutritive fiber, Teklad Test Diets; 3.07 kcal/gm), 33% carbohydrate (dextrose, Teklad Test Diets) or 16.5% carbohydrate (dextrose) plus 16.5% nonnutritive cellulose (nonnutritive fiber, Teklad Test Diets; 3.07 kcal/gm), and 33% fat (Thrifty Maid vegetable shortening, Winn Dixie Foods Inc.; 5.90 kcal/gm) or fat (vegetable shortening) plus 16.5% nonnutritive white petroleum jelly (pretolatum, Fisher Scientific Co.; 3.90 kcal/gm). Thus, the three groups were given a high and low caloric density diet pair of one of the three basic macronutrients. The constant 33% dilution of the powdered chow complex diet was such that any given diet would provide the necessary vitamins, minerals, and diluted macronutrients for maximal health and growth (Nutrient requirements of laboratory animals, 1972). Thus, a 100% preference for any given diet would not necessitate consumption of the other diet.

Estrogen Replacement Therapy

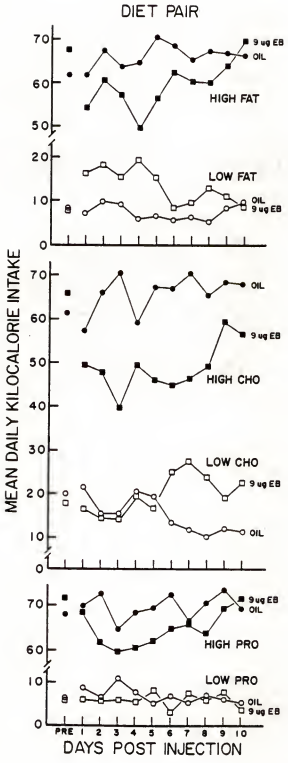
One week after each group was maintained on their respective macronutrient diets (three weeks postoperatively), each group was further divided into two groups matched for body weight and received EB injections according to one of two injection sequences, either 3, 9 ug/kg

body weight of EB and the oil vehicle or 9, 3 ug/kg body weight of EB and the oil vehicle. After the first EB injection, 11 days followed during which time the rats regained their body weights and pre-EB dietary self-selection patterns and intake. The next three days served as a baseline period for the next EB injection. After the second EB injection, an additional 11 days ensued followed by a three day pre-oil injection period. Subsequently, the rats were fed their respective maintenance diets until their body weights reached an asymtotic level (7 days with a body weight change of no more than ± 10%). At this time the animals were killed.

Results

Following OVX, a significant effect of caloric density (macronutrient concentration) was observed using a five-way ANOVA (Macronutrient X Hormone Injection Sequence X Caloric Density X Dose X Time), F (1,30) = 414.74, $p < .0001$. DNMR tests revealed that, for all three macronutrient groups, the intake of the higher caloric density diet was greater than that of the lower calorie diet (all p's < .01). This preference for the High Fat, CHO, and Protein diets can be seen in Figure 3.1 prior to either EB or oil injection (i.e., "Pre" data points). Figure 3.1 shows the mean daily kcal intake of the three diet pairs prior to and following injection of the oil vehicle and EB. Although there was a significant effect of Dose, F (2,60) = 4.59, $p < .02$, there was not a difference in caloric intake between the two doses of EB (i.e., 3 and 9 ug/kg), DNMR tests ($p > .05$). Therefore, for clarity, only the higher dose of EB is depicted. It can also be seen from this figure

Figure 3.1: Mean kcal intake of the High-Low caloric density pairs for fat, CHO, and protein diets after a s.c. injection of oil (open symbols) or 9 ug/kg EB (closed symbols).


DIET PAIR
MEAN DAILY KILOCALORIE INTAKE
9 ug EB
OIL
HIGH FAT
LOW FAT
OIL
9 ug EB
OIL
9 ug EB
HIGH CHO
LOW CHO
9 ug EB
OIL
9 ug EB
OIL
HIGH PRO
LOW PRO
OIL
9 ug EB
PRE 1 2 3 4 5 6 7 8 9 10
DAYS POST INJECTION

that the preference for the higher calorically dense diet persisted after both oil and hormone injection, albeit to a lesser extent for the week following EB injection.

Because the Caloric Density X Dose interaction was significant, F (2,60) = 6.64, p < .0025, one-way ANOVAs were performed for each separate diet of the three diet pairs to determine where this interaction was occurring. These analyses revealed that only the High CHO and Protein diets had a significant dose effect, F (2,22) = 4.62 and F (2,22) = 3.97, ps < .05, respectively. DNMR tests revealed no difference between the doses of EB for both diets but a significant suppression of the High Protein and CHO intakes (ps < .05). It should additionally be noted that the suppression of High Fat by EB was nearly significant, F (2,22) = 2.21, p > .1. In addition, although not statistically significant, 10 of 12 rats in the High-Low Fat and CHO pairs increased their intake of the lower caloric diet after EB administration.

The main ANOVA also revealed a significant Dose X Macronutrient interaction, F (4,60) = 2.54, p < .05, indicating a differential effect of hormone treatment on the caloric intake of the three macronutrient diet pairs. Simple main effects tests revealed that this differential effect of hormone on macronutrient intake was occurring with the CHO diet, F (2,20) = 10.76, p < .001. DNMR tests show that both injections of EB significantly suppressed the intake of the High CHO diet and enhanced the intake of the Low CHO diet (all ps < .05) but did not differ from each other for both caloric densities (all

ps > .1). Thus, EB administration, suppressed the intakes of the High CHO and Protein diets, and, to a lesser extent, the High Fat diet. EB administration also suppressed the intake of the High CHO diet to a greater degree than that of the other diets and significantly increased the intake of the Low CHO diet.

An additional analysis of caloric intake was performed examining these data in a relative manner. That is, the caloric intakes of all diets were examined relative to the three day pre-injection mean intake. This five-way ANOVA (Macronutrient X Hormone Injection Sequence X Caloric Density X Dose X Time) did not reveal any different significant or nonsignificant main effects or interactions than the ANOVA performed on the absolute kcal intake values and, therefore will not be discussed.

Since water intake is closely related to food intake in laboratory rats (e.g., Verplanck & Hayes, 1953), it would be expected that when food intake decreases, as was shown after EB administration above, water intake should also change or other compensatory systems be brought into action (i.e., urine excretion and/or evaporative water loss). A four-way ANOVA (Macronutrient X Hormone Injection Sequence X Dose X Time) revealed a significant effect of Macronutrient, F (2,29) = 14.70, $p < .00001$ and Dose X Food, F (4,58) = 3.88, $p < .0001$. DNMR tests revealed that more water was consumed in the High-Low Protein Group than either the High-Low Fat or CHO groups ($p > .01$) ($\bar{x}$ water intake ± SEM = 27.14 ± 3.2, 25.42 ± 4.1 and 34.84 ± 2.3 mls for the High-Low Fat, CHO, and Protein Diet pairs). The intake of water was not different between the High-Low Fat or CHO diet pairs ($p > .1$).

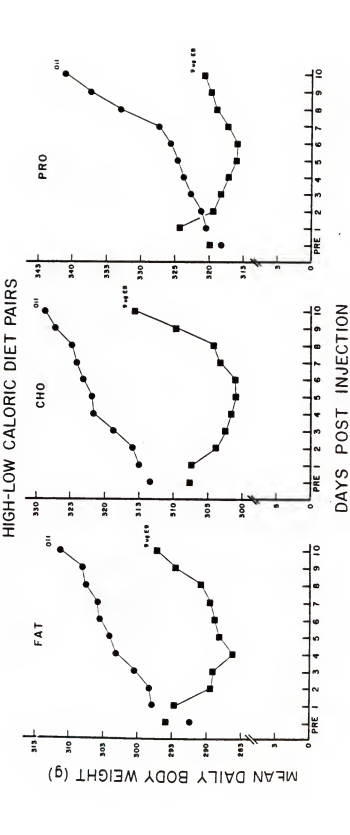
Simple main effects tests were performed to ascertain where the Dose X Food interaction was significant using one-way ANOVAs for hormone dose versus each macronutrient. These analyses resulted in a significant effect of hormonal dose for the High-Low CHO and Protein groups, F (2,33) = 6.32, $p < .01$; F (2,33) = 4.63, $p < .05$, respectively. It was then found that EB suppressed water intake for both High-Low diets ($ps < .01$) but did not do so in a dose-response manner ($ps > .1$).

The effect of EB replacement therapy on body weight was assessed using a four-way ANOVA (Macronutrient X Hormonal Injection Sequence X Dose X Time) resulting in a significant main effect of Dose, F = (2,60) = 53.71, $p < .00001$. DNMR tests show that EB injections resulted in a decrease in body weight in contrast to the vehicle injection (all $ps < .01$) but did not differ from each other. These results can be seen in Figure 3.2 where pre- and postinjection body weights are shown for each macronutrient group. Only the 9 ug/kg body weight data are depicted for clarity and because they were not different from that of the lower dose of the hormone.

The present change in body weight from the three day pre-injection mean was determined and analyzed with a four-way ANOVA (Macronutrient X Hormonal Injection Sequence X Dose X Time) but did not reveal any different significant or nonsignificant effects from that of the absolute body weight data analysis, and will therefore not be discussed.

As stated in the "Procedure" section of this experiment, all rats were allowed to continue self-selecting their food intake until their body weights reached asymtotic values, at which time they were killed.

Figure 3.2: Mean body weight after an injection of oil or 9 ug/kg EB for the High-Low Fat, CHO, and Protein diet pairs.


HIGH-LOW CALORIC DIET PAIRS
FAT
CHO
PRO
OIL
WATER
MEAN DAILY BODY WEIGHT (g)
DAYS POST INJECTION
PRE

A priori direct difference t-tests were performed on the presurgery body weights versus the body weight at the time of death. All groups gained a significant amount of body weight during this time period (all ps < .001), with the High-Low Fat, CHO, and Protein groups gaining a $\bar{x}$ ± SEM of 47.67 ± 6.34, 56.83 ± 5.76 and 82.75 ± 10.71 g, repectively. A priori independent t-tests between the means showed that the High-Low Protein diet-fed rats gained significantly more weight post-OVX than the High-Low Fat or CHO-fed rats (t (22) = 3.57, p <.01; t (22) = 2.73, p <.05, respectively). The weight gain of the High-Low Fat and CHO fed rats were not different from each other (p >.1).

Nose-anal length was measured at the time of OVX and sacrifice and analyzed within the macronutrient groups with a priori direct difference t-tests. It was found that all groups increased their length after OVX (High-Low Fat, CHO, & Protein groups, t (11) = 5.20, t (11) = 11.01, t (11) = 15.10, respectively; all ps < .01). A priori independent t-tests were additionally used to assess differences in nose-anal length due to macronutrient specific changes in skeletal growth. These analyses revealed that the increase in the $\bar{x}$ nose-anal length ± SEM of 1.93 ± .13 cm displayed by the High-Low Protein group was significantly greater than that of the High-Low Protein group was significantly greater than that of the High-Low Fat ($\bar{x}$ = 1.24 ± .25 cm) or CHO ($\bar{x}$ = 1.48 ± .14 cm) groups, t (22) = 2.55, t (22) = 3.47, ps <.01. The nose-anal lengths of the High-Low CHO and Protein fed rats did not differ significantly from one another (p >.1).

It should be noted that in this and all experiments utilizing the 9 and 3 ug/kg doses of EB, no significant dose-effect relations were found for any measure. Therefore, the 3 ug/kg data are presented in

APPENDICES A through O and labeled according to the experiment and dependent variable measured.

Discussion

The role of E2 in the dietary self-selection of the three basic macronutrients varying in caloric density was clearly shown in the results of Experiment 1. Regardless of the predominent macronutrient in the High-Low diet pairs, the removal of estrogenic stimulation by OVX produced clear preferences for the more calorically dense diet. Specifically, nearly 80-90% of all calories consumed for the three diet pairs were selected from the more calorically dense diet (i.e., Fat: 88%, CHO: 77%, & Protein: 91%). Further, although preference for the more calorically dense diet continued, EB replacement therapy significantly decreased intake of these diets while reliably but not significantly, in terms of statistics, while increasing the intake of the calorically dilute diet. Thus, it appears that one effect of E2 on the control of food intake is to affect the selection of dietary sources based on caloric density. That is, circulating E2 levels are inversely related to the selection of calorically dense diets.

It is important to note, however, that although EB replacement therapy decreased the intakes of all three calorically dense diets, it did so with some macronutrient specificity. That is, CHO intake was differentially affected by EB injection resulting in a greater suppression of the High CHO diet than that of the High Protein or Fat diets while simultaneously increasing the intake of the Low CHO diet. This special relation between E2 and CHO will continue to be seen throughout these experiments.

Injections of EB reduced body weight in all three groups of rats, regardless of the major dietary constituent. This effect was observed regardless of differences in body weight of the rats in the different groups. This is somewhat surprising in light of the data of Redick, Nussbaum, and Mook (1973) that indicated that the effectiveness of EB in reducing body weight was greater as obesity increased. Recently, Mueller and Hsiao (1981) reported that chronic EB injections were relatively ineffective in reversing the weight gain seen after OVX. However, using the Lee index (a body weight to body length ratio) as an indicant of obesity (Bernardis & Patterson, 1968; however, see Stephens, 1980), EB administration completely reduced the OVX-induced increase in body fat. The failure to completely reverse the body weight increases seen after OVX, according to Mueller and Hsiao (1981), was their "discovery" of an OVX-induced increase in skeletal growth and supporting tissues (see Freudenberger & Billeter, 1935; Grunt, 1964, Kakolewski, Cox, & Valenstein, 1968; Stotenburg, 1913; Tang, 1941; for earlier accounts of OVX-induced growth changes). It is possible, therefore, that although the High-Low Protein diet-fed rats exhibited greater body weights, this weight was not all as body fat and the reduction seen after EB injection was only to that portion of the increased body weight that was fat. Thus, although heavier, these rats were not more obese and therefore did not lose more weight than the CHO or Fat diet-fed rats. In support of the suggestion that some of the body weight increase in the High-Low Protein diet-fed rats was due to growth was their significantly larger naso-anal

lengths. Without carcass analysis data it can not be unequivocally determined as to which portions of the increase in body weight of these animals was as fat or protein. It should be noted that it has recently been demonstrated that high protein diets promote increased body weight as fat in adult male rats (Donald, Pitts, & Pohl, 1981) suggesting that consumption of a high protein diet can result in obesity and not necessarily linear growth and muscle mass increases (e.g., Edozien & Switzer, 1978). Further investigations await a more thorough understanding of OVX-induced body weight changes and their underlying changes in carcass composition in rats self-selecting their macronutrient intakes.

It is also surprising that the High-Low CHO diet-fed rats were more responsive to the suppressive effects of EB administration on caloric intake than that of the other two groups in light of the report of Young, Nance, and Gorski (1978). These workers fed groups of ovariectomized rats either a single diet identical to that of the High Fat diet of the present experiment or an identical diet to the High CHO diet. In terms of the percent change from baseline food intake, the high fat-fed rats suppressed their caloric intake to a greater degree than High-CHO fed rats following a single injection of 6 ug of EB, an intermediate dose in comparison to those used in the present experiment (i.e., 3 & 9 ug). This discrepancy may be due to one or both of two factors. First, in the present study, although most of the caloric intake was consumed from the more calorically dense diet, 10 to 20% was consumed from the more calorically dilute

diet. Total kcal intake did not vary between the rats fed the fat diet of Young et al. (1978) and the High-Low Fat diet-fed rats employed in this experiment (approximate average total kcal intake = 83 versus 75 kcal, respectively). Therefore, the opportunity to ingest food from another source may have diminished the suppression of the High Fat diet in the present study.

The second factor that may be an important difference between the results of these studies is that the total caloric intake of the CHO diet in the Young et al. (1978) study was lower than that in the present study. However, once again the presence of an alternative diet,the Low CHO diet in the present study, appears to be an important variable in the total kcal consumed. That is, there is no difference in the caloric intakes of the identical CHO diets in the two studies but in the present study an additional 20 kcal were ingested per day of the Low CHO diet. Therefore, in the present study, total kcal intake was equal between the High-Low Fat diet-fed rats and the High-Low CHO diet-fed rats, presumably due to the presence of an alternative diet, particularly the Low CHO diet. Young et al. (1978) did not have equal total kcal intakes between the fat- and CHO-fed groups resulting in different baseline food intakes with which to assess the effects of EB and, therefore, necessitating the use of the potentially artifactual relative measure, percent change (e.g., a decrease in intake of 50 to 25 kcal represents a 25 kcal difference absolutely while a reduction from 10 to 5 kcal results in a 5 kcal difference,

yet the relative percent change would be equal). The absolute suppressions of the CHO diet in the Young et al. (1978) study are not different from that of the fat diet, only the relative measure differs, perhaps compromising the conclusion that fat-fed rats are more responsive to the estrogenic suppression of food intake than are CHO-fed rats.

The increase in water intake associated with the High-Low Protein diet appears to reflect the well documented increase in water intake associated with protein intake (e.g., Fitzsimons & LeMagnen, 1965; Radford, 1959), presumably due, in the long term, to the additional requirement of water for nitrogenous secretions. This increase in water intake is seen in all of the following studies when protein is one of the members of a diet pair and therefore will not be subsequently discussed.

Water intake was found to decrease after injection of EB following a time course similar to that of food intake. This result is entirely expected since food and water intake are closely coupled in rats (e.g., Verplanck & Hayes, 1953). This suppression of water in EB injected rats is seen in all the subsequent experiments using the gonadectomy-replacement paradigm and will not be subsequently discussed.

To summarize, E2 appears to exert some control over diet selection based on caloric density but, in addition exhibits macronutrient specificity by affecting the intake of CHO to the greatest extent. Because E2 can affect diet selection based on caloric density, the

results and interpretations of previous studies employing spayed and spayed rats with E2 replacement therapy not using isocaloric diets must be questioned (Geiselman et al., 1981; Kanarek & Beck, 1980; Young et al., 1978).

CHAPTER IV

EXPERIMENT 2: ROLE OF NATURALLY-OCCURRING CHANGES IN ESTRADIOL ON MACRONUTRIENT SELF-SELECTION

The results of Experiment 1 indicate that ovariectomized rats will become obese when given diets with high concentrations of CHO, fat, or protein and, moreover, that EB replacement therapy, while suppressing the intakes of all three calorically dense diets, produces a greater suppression of the CHO diet. Although OVX and OVX plus EB replacement therapy affected the intake of all three macronutrients and a suggestion of a macronutrient specific effect of EB on the intake of CHO was found, diets representing all three basic macronutrients were not simultaneously presented. Further, Experiment 1 employed a gonadectomy-replacement paradigm and, although interesting, these results may simply reflect an endocrinological artifact. Therefore, based on the apparent need to use diets of equal caloric density and to obtain self-selection data from intact, cycling rats, the following experiment was performed using three, simultaneously available, isocaloric diets representing the three basic macronutrients.

Method

Subjects

Twelve adult, intact female rats ($\bar{x}$ body weight ± SEM = 299.1 ± 16.6 g) were presented with three isocaloric (3.73 kcal/gm) macronutrient diets presented simultaneously representing the three basic macronutrients (i.e., fat, CHO, and protein; for a complete description of the composition of these diets see Table 4.1).

Procedure

Daily food and water intake and body weight measures were monitored for 28 consecutive days. These measurements were taken prior to the onset of the dark phase of the photocycle and, in addition, a vaginal smear was taken at this time to assess estrous cycle stage in close proximity to when the majority of the ingestive behavior takes place.

Results

Nine of the twelve rats met the minimum five estrous cycle requirement. Because a "pure" metestrous cycle stage was rarely observed (i.e., a vaginal smear containing only cornified cells and leucocytes), this stage of the cycle was omitted from statistical analysis but was included in the figures for continuity

Table 4.1

Isocaloric Diet Composition

Fat				Carbohydrate			
Petroleum Jelly	18.33%	0.0	kcal/gm	Glucose	33.0%	4.0	kcal/gm
Vegetable Shortening	14.67%	9.0	kcal/gm	Purina Powdered Chow	67.0%	3.6	kcal/gm
Purina Powdered Chow	67.00%	3.6	kcal/gm		100.0%	3.73	kcal/gm
	100.00%	3.73	kcal/gm				

Protein			
Casein	33.0%	4.0	kcal/gm
Purina Powdered Chow	67.0%	3.6	kcal/gm
	100.0%	3.73	kcal/gm

purposes. Figure 4.1 displays the frequency of estrous cycles of varied lengths and the animal that is associated with each cycle length. For example, it can be seen in Figure 4.1 that Rat 57 displayed two 4-day, two 5-day, and one 6-day estrous cycle. The $\bar{x}$ number of estrous cycles ± SEM displayed by these nine rats was 6.10 ± .45, with the $\bar{x}$ ± SEM cycle length being 4.37 ± .16 days.

A two-way ANOVA (Macronutrient Diet X Estrous Cycle) was used to evaluate the kcal intake of the three diets representing the three basic macronutrients across three of the estrous cycle stages (i.e., proestrus, estrus, and diestrus).

This analysis indicated a significant effect of the various diets on caloric intake, F (2,16) = 6.86, p < .01, and moreover, a significant Macronutrient Diet X Estrous Cycle interaction, F (4,32) = 54.90, p <.00001. Simple main effects were employed to determine where the interaction of ovarian hormonal status and macronutrient intake occurred (i.e., one-way ANOVAs for each of the diets examining caloric intake across the estrous cycle stages). Only the analysis of CHO and Fat intake were statistically significant, F (2,16) = 33.97, p < .00001; F (2,16) = 42.58, p < .00001, respectively. However, it should be noted that six of the nine rats decreased protein intake during estrus and seven of the nine rats increased protein intake during diestrus, for every estrous cycle. The left half of Figure 4.2 graphically displays these data and that of the metestrous stage. It should also be noted that five of the nine rats displayed at least one metestrous smear and that the mean caloric intake during

Figure 4.1: Frequency of estrous cycle length in days. Each number represents an estrous cycle for the rat assigned that identification number.

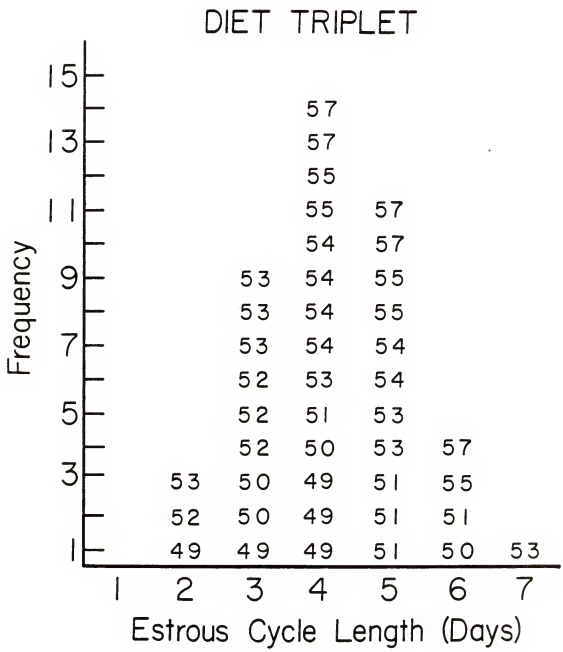
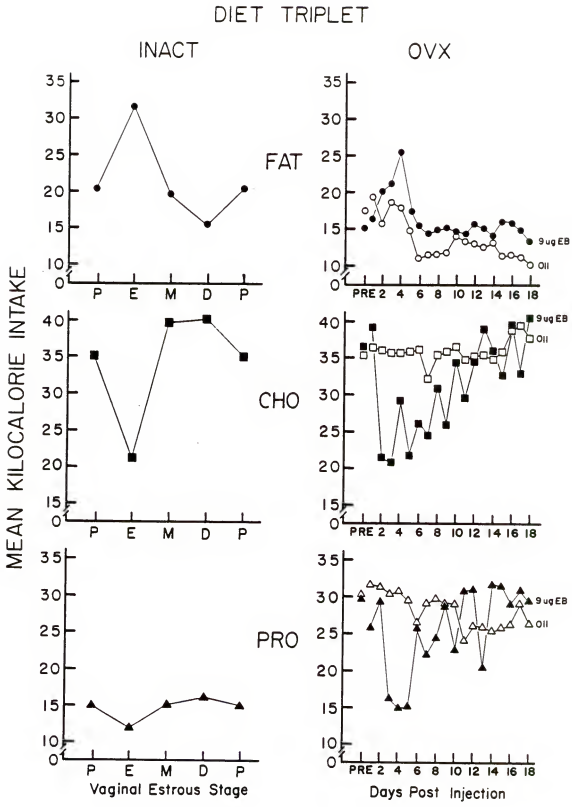

DIET TRIPLET
Frequency
15
13
11
9
7
5
3
1
57
57
55
55 57
54 57
53 54 55
53 54 55
53 54 54
52 53 54
52 51 53
52 50 53 57
53 50 49 51 55
52 50 49 51 51
49 49 49 51 50 53
1 2 3 4 5 6 7
Estrous Cycle Length (Days)

Figure 4.2: Mean kcal intake for each macronutrient diet of the diet triplet for intact rats (left half of figure) across the estrous cycle (P = proestrus, E = estrus, M = metestrus, & D = diestrus) and ovariectomized (right half of figure) rats given s.c. injection of oil (open symbols) or 9 ug/kg EB (closed symbols).


DIET TRIPLET
INACT
OVX
FAT
CHO
PRO
MEAN KILOCALORIE INTAKE
P E M D P
PRE 2 4 6 8 10 12 14 16 18
9 ug EB
Oil
Vaginal Estrous Stage
Days Post Injection

this stage of the estrous cycle, based on 13 smears, is depicted in this figure, in contrast to a minimum of 45 smears for the other estrous cycle stages (i.e., five cycle minimum multiplied by nine rats). DNMR tests performed on the CHO and Protein data revealed that CHO and Fat intake significantly varied across all three estrous cycle stages (all *ps* at least < .05). Specifically, CHO intake was maximal during diestrus, less during proestrus, and reached its nadir during estrus. In contrast, as can be seen in Figure 4.2, the intake of fat formed a "mirror image" function to that of CHO intake with maximal intake seen during estrus, minimal during diestrus, and an intermediate level of intake seen during proestrus.

A two-way ANOVA (Macronutrient Diet X Estrous Cycle) was performed on the relative percentage of the total caloric intake for each diet at each estrous cycle stage. This analysis did not reveal any new significant or nonsignificant *F* values and therefore is not presented. However, because other studies examining the role of ovarian hormonal status and macronutrient intake have expressed their data in this form, Table 4.2 presents the percentage intakes of the three macronutrient diets across the estrous cycle, including the metestrous data. It can be seen that, during proestrus, intake of all three diets is very similar, representing approximately one-third of the total caloric intake. During estrus, about one-half of the total calories are consumed as fat with protein and CHO representing one-quarter of the total intake each. Because the metestrous data represent fewer animals and cycles, caloric intake can tentatively be

Table 4.2

Mean Percent of Total Caloric Intake ± SEM of Each Macronutrient Diet Across the Estrous Cycle

	Estrous Cycle Stage			
	Proestrus	Estrus	Metestrus	Diestrus
Fat	33.49	52.05	25.67	27.06
	±3.65	±3.40	±.85	±2.33
CHO	39.55	25.20	54.78	45.54
	±5.39	±3.74	±3.62	±4.73
PRO	20.95	22.74	19.53	27.40
	±4.16	±7.00	±1.29	±4.12

described as about one-half the intake as CHO with the other two diets representing one-fourth of the total. The percentage of total calories consumed as CHO accounts for nearly half the total caloric intake during diestrus with about one-fourth of the calories eaten as fat and protein.

A one-way ANOVA (Estrous Cycle) was conducted on the total caloric intake across the three stages of the estrous cycle statistically evaluated above. There was no significant change in the total kcal intake across the estrous cycle, F (2,16) = 0.32, $p > .7$.

A one-way ANOVA (Estrous Cycle) was used to analyze body weight changes across the estrous cycle, and revealed no significant change in body weight, F (2,16) = .01, $p > .99$.

Water intake, as influenced by estrous cycle stage, was assessed using a one-way ANOVA (Estrous Cycle) and indicated that no change in fluid consumption occurred across the cycle, F (2,16) = 1.28, $p > .3$.

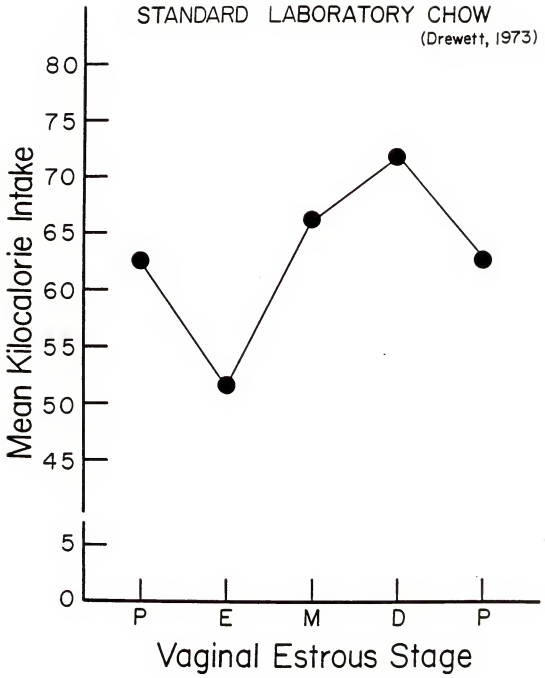
Discussion

The results of Experiment 2 suggest that E2 modulates the intake of CHO by decreasing the intake of CHO when circulating endogenous levels are high, as during estrus, and exhibiting their maximal behavior effects (ter Haar, 1972), and increasing the intake of CHO when circulating E2 levels are low (i.e., diestrus, ter Haar, 1972). In contrast to this inverse relation between CHO intake and E2 stimulation, fat intake is directly related to that of circulating E2 plasma titers. That is, fat intake is enhanced during estrus and suppressed during diestrus. Although not statistically significant the intake

of protein followed a similar pattern to that of CHO but with a much diminished amplitude. Total caloric intake was not different across the estrous cycle, apparently due to the near mirror-image functional relation between fat and CHO intake. Body weight and water intake, in turn, were also not different across the estrous cycle. Thus, it appears that E2 is not a hormone that universally depresses food intake but only exerts its anorexic effects on CHO and, to a much lesser extent, protein. In addition, E2 appears to enhance the intake of dietary fat. These resultant increases and decreases in macronutrient intake do not affect total caloric intake and, under these conditions, body weight.

At first, these data appear to be incongruent with nearly 40 years of research clearly showing 1) total caloric intake is inversely related to circulating E2 titers (ter Haar, 1972) and 2) body weight covaries with food intake, decreasing during estrus and increasing during diestrus (e.g., Brobeck, Wheatland, & Strominger, 1947). Figure 4.3 is drawn from the data of Drewett (1973) and is typical of the food intake data that have been collected in intact, cycling rats. If the shape of the relation between vaginal estrous cycle stage and caloric intake is compared to that for only the CHO intake in the left-middle section of Figure 4.2, it can be seen that these relations are quite similar. This is not surprising if one is made aware that commercial laboratory rat chow is over 50% CHO, 20% protein and 5% fat (e.g., Purina 5001 Laboratory Chow, 52.9% CHO, 22.8% protein and 4.5% fat). If the cycling rat responds to some

Figure 4.3: Mean kcal intake of standard laboratory chow by intact rats across the estrous cycle (P = proestrus, E = estrous, M = metestrus, & D = diestrus) drawn from the data of Drewett (1973).


STANDARD LABORATORY CHOW
(Drewett, 1973)
Mean Kilocalorie Intake
80
75
70
65
60
55
50
45
5
0
P
E
M
D
P
Vaginal Estrous Stage

aspect of the predominant macronutrient and/or even the second most abundant macronutrient, it would be predicted that the same functional relationship between vaginal estrous stage and total caloric intake of standard laboratory chow would be observed to some degree. In short, the cycling rat can only decrease its food intake during estrus and increase it during diestrus because of the lack of other dietary choices and the composition of standard laboratory chow. If given choices that represent the three basic macronutrients that are isocaloric, total caloric intake, body weight, and water intake do not vary across the estrous cycle but the source from which the calories are selected does change.

CHAPTER V

EXPERIMENT 3: DIETARY SELF-SELECTION DURING THE DEVELOPMENT OF OVARIECTOMY-INDUCED OBESITY

Since Experiment 2 demonstrated that dietary self-selection changes occur across the estrous cycle, it might be expected that removal of the ovaries would result in a shift in diet selection to one that resembles that with minimal estrogenic stimulation, diestrus (ter Haar, 1972). If the increased circulating level of progesterone seen during diestrus has effects on diet self-selection in and of itself, then some compromised form of a diestrous profile of diet selection may be obtained. This possible bona fide effect of progesterone on food intake would contradict the current view that this hormone simply negates the effects of E2 on food intake (e.g., Wade, 1976) and numerous experimental evidence indicating the same (e.g., Roberts et al., 1972; Rodier, 1971; Ross & Zucker, 1974; Wade, 1975; Zucker, 1969).

Examination of the development of the possible dietary changes seen after OVX may indicate the time course of some of the physiological consequences of this surgery. For example, as it was stated earlier, OVX has been shown to produce both an increase in body weight and an increase in skeletal growth and its associative supportive

tissues (e.g., Freudenberger & Billeter, 1935; Grunt, 1964; Kakolewski et al., 1968; Mueller & Hsiao, 1981; Stotenburg, 1913; Tang, 1941). It may be that this increase in linear growth is not a slow incremental increase. Although there are not any data on the temporal locus of the skeletal growth, these animals do not continue to gain weight indefinately (e.g., Bernardis, Bellinger, Goldman, & MacKenzie, 1981) but show a rapid body weight increase followed by an asymtotic, static phase of body weight gain. The increase in skeletal growth may be restricted, therefore, and reflected in an increase in protein intake within a given time frame.

After some physiological manipulations a nearly immediate preference for a food source has been demonstrated (for review see Overmann, 1976). For example, Richter (1941) found an increase in the intake of sodium chloride within hours after adrenalectomy. In contrast, rats made diabetic by partial pancreatectomy (Richter, Schmidt, & Malone, 1945) gradually increased protein and fat intake while CHO intake slowly decreased. While the rapidity within which one observes changes in diet selection has been argued as a way of discerning whether there is an innate "specific hunger" for a particular substance (see Booth, 1979, for a treatment of this issue). The ability of certain diet preferences to have a "learned or acquired" aspect for their selection has been clearly shown in the work of Booth pairing flavor/odor with preferred protein diets in protein-deprived and diabetic rats. These rats were then presented with nutritionally

equivalent diets with the various flavor/odors and demonstrated strong preferences for the protein flavor/odor diets (Booth, 1974; Booth & Simson, 1971; Simson & Booth, 1973, 1974). Although the rapidity of a dietary preference seems more likely to represent the degree to which the animal is challenged, therefore making learned versus innate conclusions not possible, it would be interesting to note the development of dietary choice changes beginning immediately following OVX to assess the severity of the consequences of the removal of the ovaries on diet selection and caloric intake.

The following experiment was designed to assess the development of dietary change following OVX using the rats from Experiment 2 that have had an extensive history of self-selecting their intakes from these diets to avoid possible novelty affects from obscuring choice changes.

Method

Subjects

Twelve adult female rats that began Experiment 2 had a mean body weight of 312 ± 17.8 g and received bilateral ovariectomies as described above.

Procedure

Because these animals had an extensive history of self-selecting the equal caloric macronutrient diet triplet in Experiment 2, the measurement period for these animals began immediately postoperatively.

This early measurement period, in contrast to the two week delay that occurred for the ovariectomized rats employed in Experiment 2, allowed for the observation of the development of OVX-induced obesity and possible changes in dietary self-selection as this development occurred.

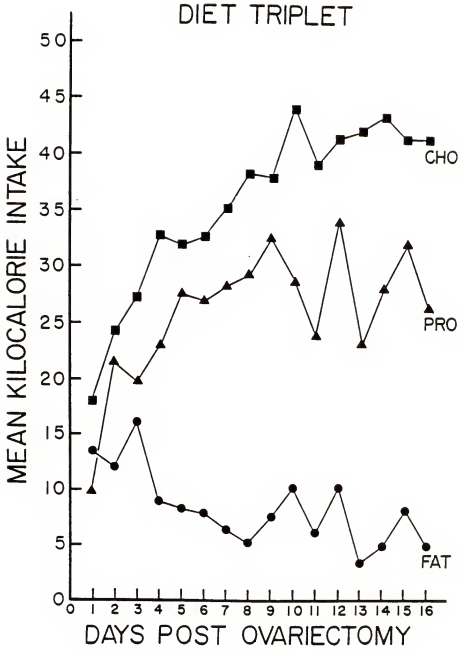
Results

The effects of OVX on dietary self-selection during the development of obesity were examined for kcal intake using a two-way ANOVA (Macronutrient X Time). There was a significant effect of OVX on macronutrient intake, F (2,22) = 9.88 $p < .001$. It was further found that the caloric intake of CHO was greatest during the developing obesity, protein intermediate, and fat intake the smallest (DNMR test, all $ps < .01$).

These data are graphically displayed in Figure 5.1 where the mean kcal intake of the three isocaloric diets is depicted as a function of days post-OVX. It can be seen from this figure that after removal of the ovaries, the intake of CHO and protein increases while that of fat progressively decreases, with the intake of all diets relatively stable one week after surgery.

The relative change in the caloric intake of the three diets was examined as a function of time since ovarian hormonal withdrawal using three, one-way ANOVAs (Time), one for each macronutrient. The percent change in caloric intake relative to the first day following ovariectomy was used to avoid a potential bias by the greater representation of one stage of the estrous cycle if the intakes from the day prior to surgery were used. The percent change in caloric intake

Figure 5.1: Mean kcal intake of each macronutrient of the diet triplet (CHO = squares, Protein = triangles, & Fat = circles) following ovariectomy.


DIET TRIPLET
MEAN KILOCALORIE INTAKE
50
45
40
35
30
25
20
15
10
5
0
CHO
PRO
FAT
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
DAYS POST OVARIECTOMY

for CHO was significant, F (14,154) = 2.10, $p < .05$; ranging from a $\bar{x}$ ± SEM of 156.71 ± 27.27% on the second day after surgery to 263.21 ± 48.06% on the last day. Although Figure 3.1 shows a similar change for protein intake, the percent change in caloric intake was not significant, F (14,154) = 1.03, $p > .4$. Close inspection of this figure reveals that by the fifth day-post OVX, the intake of protein was nearly maximal and thus, although over a 300% change in protein intake was observed, this change did not significantly vary across time. The percent change in fat intake was also significant, F (14,154) = 3.33, $p < .0001$, decreasing across time from a $\bar{x}$ ± SEM on the second day after OVX of 97.36 ± 18.88 to 32.63 ± 16.68 on the last day. Thus, both the percent change in the caloric intake of CHO and protein increased post-OVX, albeit at different rates, while that of the fat diet decreased.

Total caloric intake increased as a function of time since OVX (one-way ANOVA, Time), F (15,165) = 15.73, $p < .00001$, nearly doubling across the time period examined ($\bar{x}$ total caloric intake ± SEM: Day 1 = 41.65 ± 3.75, Day 16 = 72.42 ± 3.88). However, it should be noted that if the average daily caloric intake is computed across the estrous cycle for these same rats, a total caloric intake of about 68 kcal/day is obtained. Since it was shown in the previous experiment that total caloric intake did not change across the estrous cycle in these rats, allowing for an average total kcal intake to be used, the low caloric intakes immediately following OVX appear to be due to a trauma-related effect of surgery and, therefore the total caloric

intake seen after about Day 4, approximately 60 kcal, does not appear to change appreciably during the next 12 days of observation. Thus, the significant increase in total caloric intake as a function of time since OVX may be an artifact of postsurgical-trauma.

Water intake was also found to be significantly affected by OVX across time, (one way ANOVA, Time), F (15, 165) = 3.35, $p < .0001$, ranging from a $\bar{x}$ ± SEM of 26.75 ± 2.91 ml on the first day after surgery to 33.08 ± 2.18 ml on the last day. Table 5.1 shows the $\bar{x}$ ± SEM water intake for the 16 day measurement period following OVX and it can be seen that, similar to the total kcal intake data, water intake begins quite low but by Day 3 or 4 overlaps with most of the subsequent water intakes. Once again, since water intake did not vary with estrous cycle stage in these same rats and averaged approximately 32 ml across the estrous cycle, allowing for a mean water intake to be legitimately used, the significant change in water intake post-OVX seems to reflect an effect of surgical trauma, not a bona fide effect of the removal of ovarian hormonal stimulation.

Body weight was analyzed post-OVX using a one-way ANOVA (Time) and found to be significant, F (15, 165) = 52.69, $p < .00001$. This significant effect of OVX on body weight does not appear to be artifactual, however. In these same rats, body weight did not change across the estrous cycle and averaged about 312 g. Table 5.2 shows the $\bar{x}$ ± SEM body weight of these rats following OVS for the 16 day observation period. It can be seen that, although a one to three day

Table 5.1

Mean Daily Water Intake ± SEM (ml) Following Ovariectomy

Day Post Ovariectomy	Mean Water Intake ± SEM (ml)
1	26.75 ± 2.91
2	27.33 ± 2.63
3	28.58 ± 1.50
4	30.33 ± 1.77
5	32.50 ± 1.87
6	34.08 ± 2.92
7	29.05 ± 3.10
8	32.82 ± 3.69
9	32.83 ± 2.55
10	32.67 ± 2.17
11	28.58 ± 1.75
12	36.25 ± 2.71
13	27.58 ± 1.88
14	30.92 ± 2.37
15	28.42 ± 2.18
16	33.08 ± 2.18

Table 5.2

Mean Daily Body Weight ± SEM (g) Following Ovariectomy

Days Post Ovariectomy	Mean Body Weight ± SEM (g)
1	309.17 ± 5.47
2	310.75 ± 5.48
3	313.25 ± 5.33
4	315.83 ± 5.27
5	316.92 ± 5.22
6	319.08 ± 5.30
7	317.92 ± 5.07
8	321.17 ± 5.16
9	322.00 ± 5.33
10	326.00 ± 5.18
11	329.42 ± 5.22
12	331.58 ± 5.19
13	332.25 ± 5.27
14	333.58 ± 5.16
15	337.33 ± 5.32
16	340.00 ± 5.43

decrease in body weight occurred following removal of the ovaries, this presumed surgical trauma-induced suppression in body weight was followed by a rapid weight gain reaching a $\bar{x} \pm$ SEM body weight of 340 ± 5.43 g by Day 16 or, an increase of about 28 g from their intact body weights (approximately a 2.3 g increase per day in contrast to a maximal change of .2 g across the estrous cycle).

Discussion

Removal of the ovaries resulted in a progressive increase in CHO and protein intake while fat intake simultaneously decreased. This resultant pattern of macronutrient selection is similar to that during diestrus (see Figure 4.2). While both diestrus and OVX are associated with low or absent E2 it would be naive to assume that OVX simply produces a physiological and behavioral state of diestrus.

Concurrent with the changing macronutrient self-selection pattern were increased total caloric intake, water intake, and body weight. Because the significant increases in total caloric and water intake appear to be due to the postsurgical suppression of these measures with a subsequent recovery, changes in these measures appear to be artifactual and not bona fide effects of a lack of ovarian hormonal stimulation. However, although there was a four day decrease in body weight following surgery, these rats gained over 28 g during the next 12 days. This is in contrast to a .2 g maximal change in body weight of these same rats across any two estrous cycle stages. Thus, the increase in body weight (2.3 g/day) seems to be due to the removal of the ovaries and is a well documented effect under conditions of a maintenance diet of standard laboratory rat chow (for review, see Wade, 1972).

The increase in body weight without an increase in total caloric intake is not without precedent. Roy and Wade (1977), Landau and Zucker (1976), and Mueller and Hsiao (1980) all have demonstrated that ovariectomized rats will show considerable increases in body weight with restricted food intake. Moreover, Geiselman et al. (1981) reported that ovariectomized rats self-selecting their caloric intake from purified macronutrient sources did not increase their caloric intakes despite increased body weight in comparison to a group of intact, cycling female rats. Thus, it appears that increased food intake is not a prerequisite for OVX-induced obesity. The most likely means by which such increases in body weight could occur, independent of increases in food intake, is a decrease in energy expenditure, perhaps as locomotor activity (for review see Wade, 1972). Alternatively, some as yet unidentified metabolic effect may underly the OVX-induced increase in body weight without an increase in food intake. One such mechanism that has been suggested is a decrease in the thermogenic activity of brown adipose tissue (Wade, Note 1).

The changes in diet selection began quite rapidly after OVX but did demonstrate a slow incremental change with respect to CHO and, to a lesser degree, protein intake and a longer lasting decremental change in the intake of fat. Because these changes can be seen in the individual intakes for these animals, the progressively changing diet selection is not due to the averaging of the data from individual rats. It is tempting to speculate that the immediate trend toward

the resultant enhanced CHO and protein intake, and decreased fat intake was due to a dissipating effect of the ovarian hormones, particularly E2, on nervous and peripheral tissues after the removal of the ovaries. However, the results of some current work tend to refute this view. That is, the same progressive changes in diet selection are seen four weeks after OVX when the rats are first presented with these diets (Bartness, Note 2). It seems unlikely that these rats would still be influenced by the ovarian hormones whose source was removed one month earlier. In contrast, it seems more reasonable to speculate that the postingestive consequences of these diets are reinforcing, in the cases of CHO and protein, and aversive, in the case of fat. What the unconditioned stimulus, reinforcer or aversive consequence may be are, of course, unknown.

These data also indicate that OVX does not simply produce a functional state of diestrus, as was also noted by Drewett (1973) in response to the increase in caloric intake seen with a maintenance diet of standard rat chow, but produces a greatly enhanced intake of protein and greater suppression of dietary fat. It may be that the lack of high circulating levels of progesterone after OVX that accompanies diestrus (for review see Feder, 1981) is responsible for these changes. Research in progress at this writing is examining a possible effect of progesterone per se on the dietary selection patterns using these diets (Bartness, Note 3). It may have been that previous studies showing no effect of progesterone on food intake resulted from the employment of standard rat chow, a diet high in CHO content.

CHAPTER VI

EXPERIMENT 4: ROLE OF EXPERIMENTALLY-INDUCED ESTRADIOL ADMINISTRATION ON MACRONUTRIENT SELF-SELECTION

The results of Experiment 2 clearly demonstrate that macronutrient self-selection varies across the estrous cycle with the changes in diet selection correlated with data for the waxing and waning of E2 plasma titers (ter Haar, 1972). Although, as stated previously, E2 appears to be the ovarian hormone responsible for changes in caloric intake seen across the estrous cycle and that the changes in caloric intake seen during estrus can be mimicked in ovariectomized rats receiving E2 replacement therapy (for review see Wade & Gray, 1979), it remains to be shown whether the macronutrient self-selection data of Experiment 2 for estrus can be mimicked by E2 replacement therapy. The following experiment was performed to test the role of E2 on dietary self-selection more explicitly than can be inferred from the data of intact rats in Experiment 3.

Method

Subjects

The twelve adult female rats used in Experiment 2 served as subjects after the 16 day post-OVX observation period was completed.

Procedure

The twelve rats were further divided into two groups matched for body weight and received subcutaneous injections of EB according to the two injection sequences used in Experiment 1 (i.e., 3, 9 ug/kg body weight, oil, or 9, 3 ug/kg body weight, oil). As in Experiment 1, a postinjection period occurred only it was 18 days in length, during which time the rats regained their lost body weight and pre-EB dietary self-selection patterns and intakes. Similarly, the next three days served as a baseline period for the next EB injection. After the second EB injection, an additional 18 days ensued, followed by a three day pre-oil injection period and an 18 day post-oil injection period.

Results

The caloric intake of the three macronutrient diets as affected by estradiol replacement was analyzed with a four-way ANOVA (Hormonal Injection Sequence X Macronutrient Diet X Dose X Time). There was a significant main effect of diet, F (2,20) = 6.41, p < .05. Moreover, the caloric intakes of all three macronutrients were different from one another (DNMR test, all ps < .01). Specifically, the intake of the CHO diet was greatest, that of protein intermediate, and fat intake smallest.

Estradiol was found to differentially affect macronutrient intake (i.e., Macronutrient Diet X Dose interaction), F (4,40) = 7.42, p < .001. Three, one-way ANOVAs (i.e., simple main effect tests) were

performed to assess where the interaction of hormone and macronutrient occurred. Estradiol significantly altered caloric intake of all macronutrients, CHO: F (1,33) = 7.92, $p < .01$; protein: F (1,33) = 4,61, $p < .05$ and fat: F (1,33) = 5.51, $p < .05$. Specifically, both doses of EB increased fat intake (DNMR tests, both $ps < .01$) but did not differ from each other in this respect (DNMR test, $p > .1$). The caloric intake of both the CHO and protein diets was suppressed by both doses of EB (DNMR test, $p < .01$) however, once again there was no difference in caloric intake between the 3 ug and 9 ug/kg EB doses (both $ps > .1$). Thus, EB injections suppressed CHO and protein while enhancing fat intake. These results are shown in the right half of Figure 4.2. Since both doses of EB did not differ with respect to their effect on the intake of all three macronutrients, for clarity, only the 9 ug dose is shown in relation to the oil vehicle both pre- and postinjection.

The effects of EB administration on the relative caloric intake for the three macronutrient diets were performed by calculating the percent change in kcal intake in relation to the three day mean preinjection baseline. Three, two-way ANOVAs (Dose X Time), one for each diet, revealed no nonsignificant or significant effects than were seen in the analysis of absolute kcal intake. Therefore, the results of these analyses will not be presented.

The effects of EB replacement therapy on total kcal intake resulted in a significant effect of this treatment (three-way ANOVA, Hormonal Injection Sequence X Dose X Time, F (2,20) = 6.43, $p < .01$).

Table 6.1

Mean Daily Water Intake ± SEM (g)
Following Injections of EB or Oil Vehicle

Treatment	Days Post Injection	Mean Water Intake ± SEM (ml)
Oil	1	32.17 ± 1.87
	2	31.17 ± 1.44
	3	32.00 ± 1.71
	4	33.00 ± 1.97
	5	26.50 ± 1.16
	6	27.17 ± 1.43
	7	26.58 ± 1.77
	8	27.17 ± 1.34
	9	26.72 ± 1.37
	10	28.25 ± 1.86
	11	27.67 ± 1.94
	12	28.83 ± 1.97
	13	29.00 ± 2.13
	14	28.08 ± 2.11
	15	29.00 ± 2.34
	16	28.75 ± 2.11
	17	29.67 ± 2.38
	18	29.67 ± 2.06
3 ug/kg EB	1	27.92 ± 2.85
	2	23.25 ± 1.73
	3	29.17 ± 3.14
	4	24.92 ± 3.75
	5	24.58 ± 2.96
	6	28.67 ± 2.42
	7	29.17 ± 2.72
	8	28.58 ± 1.71
	9	27.08 ± 1.56
	10	28.25 ± 1.99
	11	29.25 ± 1.49
	12	27.25 ± 1.74
	13	29.25 ± 1.66
	14	29.00 ± 1.98
	15	30.00 ± 2.47
	16	31.15 ± 2.57
	17	32.25 ± 2.59
	18	27.33 ± 7.99

Table 6.1--Continued.

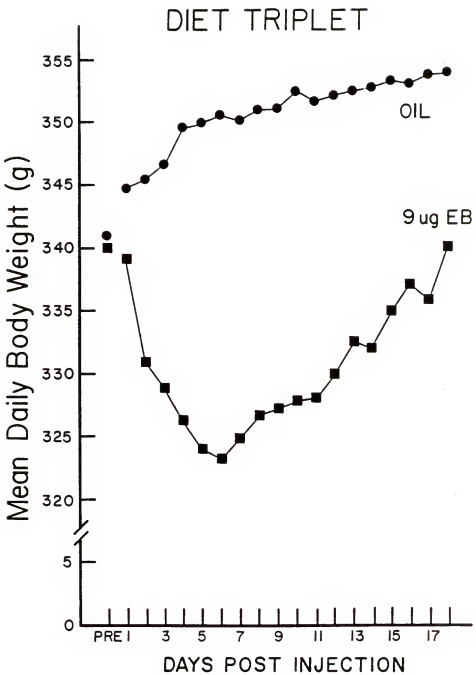
	Days Post Injection	Mean Water Intake ± SEM (ml)
9 ug/kg EB	1	31.58 ± 2.54
	2	30.50 ± 2.56
	3	26.42 ± 2.25
	4	23.83 ± 1.68
	5	22.25 ± 2.10
	6	24.92 ± 2.52
	7	30.58 ± 2.37
	8	28.33 ± 2.62
	9	30.08 ± 1.82
	10	27.17 ± 1.55
	11	28.92 ± 1.47
	12	28.75 ± 1.61
	13	29.00 ± 1.86
	14	29.25 ± 1.88
	15	29.00 ± 2.63
	16	32.00 ± 2.56
	17	28.17 ± 1.54
	18	32.00 ± 3.06

Both doses of EB significantly decreased total caloric intake (DNMR test, all $ps < .05$) but there was no difference between the suppressions produced by the two EB doses ($p > .1$).

Water intake was also affected by EB injection as seen in the results of a three-way ANOVA (Hormonal Injection Sequence X Dose X Time). Table 6.1 lists the mean daily water intake as a function of days postinjection of oil or the two doses of EB. It can be seen that the oil injection did not alter water intake but that both doses of EB caused a suppression of water intake that corresponds to the changes in body weight (see Figure 6.1) and food intake (see Figure 4.2) with the maximal suppressions occurring between three and five days post-EB injection.

Hormone injection also was found to affect body weight (two-way ANOVA, Dose X Time, $F(2,20) = 40.44$, $p < .00001$). DNMR test revealed that EB decreased body weight (both $ps < .01$) but that the two doses of EB did not differ in their effect on this measure ($p > .1$). Figure 6.1 shows the effect of EB and oil vehicle injection on daily body weight pre- and postinjection. Because the two doses of EB did not differ in respect to their suppression of body weight, for clarity, only the 9 ug/kg dose is shown. It can be seen that EB injection results in a rapid drop in body weight followed by a slower recovery while oil injection allows the slow incremental increase in body weight to proceed unabated.

Figure 6.1: Mean daily body weight (g) for ovariectomized rats given a s.c. injection of oil (circles) or 9 ug/kg EB (squares).


DIET TRIPLET
Mean Daily Body Weight (g)
355
350
345
340
335
330
325
320
5
0
OIL
9 ug EB
PRE 1
3
5
7
9
11
13
15
17
DAYS POST INJECTION

Discussion

These results indicate that EB replacement therapy produces a dietary self-selection pattern that is very similar to that seen during estrus in these same animals. That is, EB injection resulted in an increase in fat intake and decreases in the intake of CHO and protein. Once again, it would be naive to equate an EB injection with estrus, however, manipulation of this hormone is sufficient to produce the same dietary self-selection pattern as that seen during estrus. An exception to the EB replacement therapy-estrus comparison is the significance of the protein decrease after EB injection. It should be recalled that, although reliable, a small nonsignificant decrease in protein intake was observed during vaginal estrus. However, following OVX, injection of EB markedly suppressed protein intake. The difference in these findings may be due to a "floor-effect" seen in the intact, cycling rat. That is, protein intake in these animals was quite low when they were intact and perhaps the failure to observe a naturally-occurring suppression of protein at estrus was due to a level of protein intake that could not be reduced without compromising the animals health. After OVX, protein intake doubled and perhaps, with this increase, subsequent suppression by EB was more readily observable and was able to be manifested without adversely affecting the animal. Evidence for this view may be seen in the suppression of the same diet in Experiment 1 after EB injection when OVX also produced a greater consumption of this diet than that seen in the intact rats in Experiment 2.

An additional exception between the effects of estrus and those of an EB injection in ovariectomized rats is the failure of the gonadectomy-replacement procedure to alter diet selection in such a way as to maintain total caloric intake. With the two doses, of EB employed in the present study the increase in fat intake is similar to that seen in estrus as is the suppression of CHO. However, because protein intake is dramatically suppressed by EB in OVX rats, in contrast to intact rats in estrus, total caloric intake of the spayed rats injected with EB results in a suppression of total calories and, in addition, most likely accounts for the significant decrease in body weight (see Figure 6.1) and water intake.

CHAPTER VII

EXPERIMENT 5: RESTRICTION OF DIETARY CHOICE AND THE ROLE OF NATURALLY-OCCURRING CHANGES IN ESTRADIOL ON MACRONUTRIENT SELF-SELECTION

Experiment 2 demonstrated that intact, cycling rats given access to three isocaloric diets do not alter their total caloric intake across the estrous cycle but do change their dietary self-selection pattern. Further corroboration of these findings in intact, cycling rats given diet pairs composed of combinations from the diet triplet would be compelling and demonstrate the robustness of the relations between E2 and the intake of the various macronutrients. For example, it would be predicted from the diet triplet data of Experiment 2 that if a Fat-CHO diet pair were presented, during estrus fat intake should increase and CHO intake decrease with the reverse pattern seen during diestrus. Further, if a Protein-CHO diet pair were presented, both diet intakes should decrease during estrus with the reverse pattern seen during diestrus. The following experiment was conducted to examine the generality of the findings of Experiment 2 for the relations between naturally-occurring changes in estrogenic stimulation and diet selection.

Method

Subjects

Twelve adult intact female rats were given the following sequence of presentations of equal caloric (3.73 kcal/gm) diet pairs: Fat-CHO,

Fat-Protein, and Protein-CHO. For a complete description of the composition of these diets see Table 4.1. Daily vaginal smears indicated that two of the rats displayed acyclic estrous cycles. These ten animals began the first diet pair (i.e., Fat-CHO) of the sequence of diet pairs with a mean body weight of 284.2 ± 10.2 g, a weight not different from that of the three pre-OVX groups of Experiment 1. Each diet pair was presented for 28 days.

Results

Ten of the twelve rats met the minimum requirement of five estrous cycles within each 28 day presentation for all diet pairs. A clear metestrous cycle stage was also rarely observed in these rats, similar to that of Experiment 2. Therefore, this stage of the estrous cycle was omitted from the statistical analyses but was included in the figures for continuity purposes. Figure 7.1 displays the frequency of estrous cycles of varied lengths and the animal that is associated with each cycle length. For example, Rat 40 exhibited two 3-day, three 4-day and two 5-day estrous cycles for the Fat-CHO diet pair. Table 7.1 describes the $\bar{x}$ number of estrous cycles ± SEM and the $\bar{x}$ cycle length in days ± SEM for each diet pair. It should be noted that both measures overlap to a large degree.

The effect of naturally-occurring fluctuations in the ovarian hormones on dietary self-selection was examined for the three diet pairs (i.e., Fat-CHO, Fat-Protein, Protein-CHO) across the estrous cycle (three, two-way ANOVAs, Macronutrient Diet X Estrous Cycle

Figure 7.1: Frequency of estrous cycle length for the Fat-CHO, Fat-Protein, and Protein-CHO diet pairs. Each number represents an estrous cycle for the rat assigned that identification number.

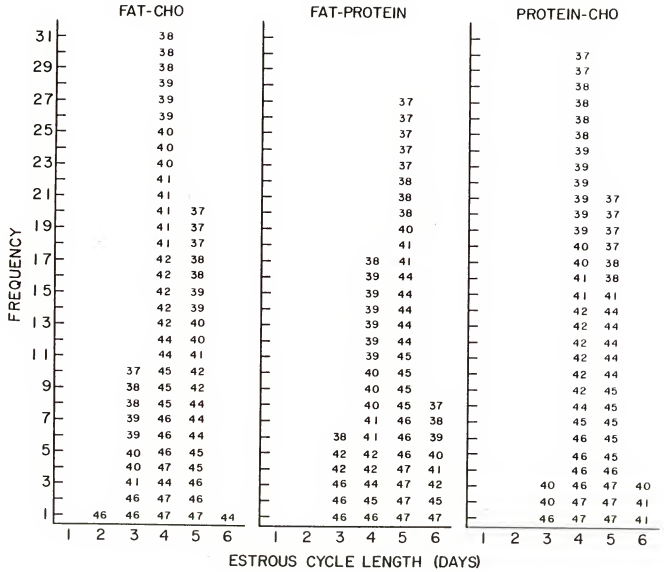

FAT-CHO
FAT-PROTEIN
PROTEIN-CHO
FREQUENCY
ESTROUS CYCLE LENGTH (DAYS)

Table 7.1

Summary of the Estrous Cycle Data for the Rats
Presented with the Sequence of Isocaloric Diet Pairs

	Diet Pair		
	Fat-CHO	Fat-Protein	Protein-CHO
Number of Complete Cycles	6.4 ±.23	6.0 ±.22	5.3 ±.35
$\bar{X}$ Cycle Length (in days) ± SEM	4.66 ±.37	4.75 ±.13	4.86 ±.28

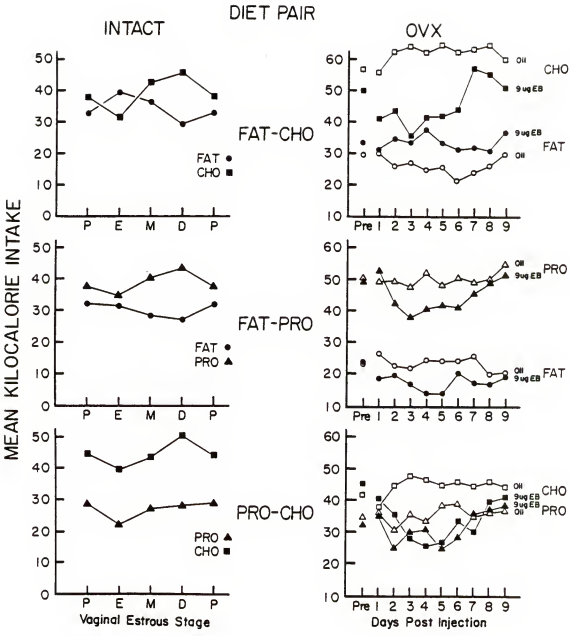
Stage). Caloric intake of the Fat-CHO diet pair varied across the estrous cycle as a function of diet (i.e., Macronutrient Diet X Estrous Cycle Stage interaction), F (2,18) = 15.41, p <.00001. Simple main effects tests (two, one-way ANOVAs, Estrous Cycle Stage, one for each diet) revealed differential intake of both diets across the estrous cycle, FAT: F (2, 18) = 6.43, p < .01; CHO: F (2, 18) = 18.49, p < .00001. Specifically, fat intake was maximal during estrus, minimal during diestrus (DNMR test, both ps < .01) but diestrous versus proestrous and proestrous versus estrous caloric intakes did not differ significantly (both ps >.1). The intake of CHO exhibited an opposite relation to estrous cycle stage. That is, CHO intake was suppressed during estrus and enhanced during diestrus (DNMR test, both ps < .01). Similar to that for fat intake, the proestrous and diestrous, and proestrous and estrous intakes of CHO were not different (both ps >.1).

The kcal intake of the Fat-Protein diet pair was examined across the estrous cycle (two-way ANOVA, Macronutrient Diet X Estrous Cycle Stage). It was found that the caloric intake of the macronutrient diets varied across the estrous cycle (i.e., Macronutrient Diet X Estrous Cycle Stage), F (2,18) = 3.49, p <.05. Further it was found that the caloric intake of fat did not significantly vary across the estrous cycle (one-way ANOVA, Estrous Cycle Stage), F (2,18) = 1.54, p > .2. However, protein intake did vary with the estrous cycle stage (one-way ANOVA, Estrous Cycle Stage), F (2,18) = 3.49,

$p < .05$. Subsequent analysis revealed that protein intake was greater during diestrus than estrus (DNMR test, $p < .01$), but that proestrous intakes of protein were not different from each other (both $ps > .1$).

The caloric intake of the Protein-CHO diet pair (two-way ANOVA, Macronutrient Diet X Estrous Cycle Stage) revealed that the intakes of the two diets differed and that there was a significant effect of the stage of the estrous cycle, Macronutrient Diet: $F(1,9) = 6.07$, $p < .05$; Estrous Cycle Stage: $F(2,18) = 17.58$, $p < .0001$. There was not a significant Macronutrient Diet X Estrous Cycle Stage interaction, $F(2,18) = 1.30$, $p > .3$. It was further discovered that the intake of CHO was greater than that of protein (DNMR test, $p < .01$) and that caloric intake was significantly less at estrus than diestrus or proestrus (DNMR test, both $ps < .01$). Proestrous and diestrous caloric intakes did not differ (DNMR test, $p > .1$). Therefore, because there was no significant Macronutrient Diet X Estrous Cycle Stage interaction this implies that the caloric intake of the two diets covary. Because the caloric intake was lowest at estrus and higher at proestrus and diestrus, both protein and CHO intake must reach their nadir at estrus and increase at the other two stages. These results and those for the Fat-CHO and Fat-Protein diet pairs can be seen graphically in the left half of Figure 7.2. In summary, if CHO is one of the diets in a diet pair, than the caloric intake of CHO is suppressed at estrus and enhanced during diestrus, as is protein. If a diet pair contains fat as one of its members, then the intake of fat will be suppressed during diestrus and increased during estrus if paired with

Figure 7.2: Mean kcal intake for each diet of the Fat-CHO, Fat-Protein, and Protein-CHO diet pairs for intact rats (left half of figure) across the estrous cycle (P = proestrus, E = estrus, M = metestrus, & D = diestrus) and ovariectomized rats (right half of figure) given a s.c. injection of oil (open symbols) or or 9 ug/kg EB (closed symbols).


DIET PAIR
INTACT
OVX
MEAN KILOCALORIE INTAKE
FAT-CHO
FAT-PRO
PRO-CHO
FAT
CHO
PRO
P E M D P
Pre 1 2 3 4 5 6 7 8 9
OH
9ug EB
Vaginal Estrous Stage
Days Post Injection

CHO. Because the intake of CHO and protein both show the same relation to estrous cycle stage, if the other diet is fat then it would be expected that their intakes both decrease during estrus and increase during diestrus, which was found.

The relative intake of each diet in terms of the total caloric intake for a given diet pair across the different estrous cycle stages was analyzed using two, one-way ANOVAs for each diet pair (Estrous Cycle). These analyses were performed to allow comparisons between diet pairs having a common macronutrient diet and between-experiment comparisons within this series of studies (i.e., diet triplet of Experiment 2). No new significant or nonsignificant effects were found as a result of these analyses that were not found for the absolute analyses. Therefore, the statistical findings of these analyses will not be reported (however, Table 7.2 describes the percentage caloric intakes of the diets across the estrous cycles stages including the metestrous stage that was not part of the statistical analysis).

The total kcal intake for each diet pair was examined as a function of the estrous cycle stage (three, one-way ANOVAs, Estrous Cycle) and found not to change significantly across the cycle for the Fat-CHO and Protein-CHO diet pairs, $\underline{F}$ (2,18) = 1.37, $\underline{p} > .3$; $\underline{F}$ (2,18) = 1.27, $p > .3$; respectively. Total caloric intake did change for the Protein-CHO diet, $\underline{F}$ (2,18) = 17.58, $\underline{p} < .0001$. It was further found that the total caloric intake was greater during diestrus than estrus (DNMR test, $\underline{p} < .01$), greater during proestrus than estrus ($\underline{p} < .01$) and not different between diestrus and proestrus ($\underline{p} > .1$).

Table 7.2

Mean Percent of Total Caloric Intake ± SEM for Each Macronutrient Diet of the Three Macronutrient Diet Pairs Across the Estrous Cycle

Diet Pair	Diet	Vaginal Estrous Cycle Stage	Mean Percent Caloric Intake ± SEM
Fat-CHO	Fat	Proestrus	46.51 ± 3.61
		Estrus	54.59 ± 2.19
		Metestrus	45.75 ± 1.63
		Diestrus	39.76 ± 2.50
	CHO	Proestrus	53.49 ± 3.61
		Estrus	45.41 ± 2.19
		Metestrus	54.25 ± 1.63
		Diestrus	60.24 ± 2.50
Fat-Protein	Fat	Proestrus	46.39 ± 6.49
		Estrus	47.05 ± 5.52
		Metestrus	41.76 ± 3.75
		Diestrus	39.76 ± 4.98
	Protein	Proestrus	53.61 ± 6.49
		Estrus	52.95 ± 5.52
		Metestrus	58.24 ± 3.75
		Diestrus	60.24 ± 4.98
Protein-CHO	Protein	Proestrus	39.19 ± 5.43
		Estrus	35.48 ± 5.94
		Metestrus	37.50 ± 4.73
		Diestrus	35.53 ± 4.97
	CHO	Proestrus	60.81 ± 5.43
		Estrus	64.52 ± 5.94
		Metestrus	62.50 ± 4.73
		Diestrus	64.47 ± 4.97

Mean daily water intake did not change across the estrous cycle for any of the diet pairs (two-way ANOVA, Macronutrient Diet Pair X Estrous Cycle).

The average body weight was examined across the estrous cycle for each diet pair (Macronutrient Diet X Estrous Cycle). This analysis revealed significant main effects of Diet Pair and Estrous Cycle, F (2,18) = 47.95, $p < .00001$, F (2,18) = 4.20, $p < .05$, respectively. However, no significant Diet Pair X Estrous Cycle interaction was found, F (4,36) = 1.00, $p > .4$. It was further found that body weight was greater during diestrus than estrus (DNMR test, $p < .05$). In addition, body weight when the rats were presented the CHO-Protein diet was greater than that when these same rats were given the Fat-CHO or Fat-Protein diet pairs, regardless of the estrous cycle stage (DNMR test, both $ps < .01$).

Discussion

The findings using all three combinations of the diet singles used in the diet triplet experiment as diet pairs in the present experiment tended to corroborate those of the diet triplet study. Thus, when the Fat-CHO diet pair was presented, fat intake increased during estrus and decreased during diestrus, with CHO intake showing the opposite effect and a net result of no change in total calories consumed across the estrous cycle stages. Similarly, when the Protein-CHO diet pair was presented, CHO intake was suppressed during estrus and enhanced during diestrus.

Protein intake tended to follow the trends seen in the diet triplet condition but, because protein intake was elevated in the diet pair conditions, significant changs in the intake of this macronutrient were seen across the estrous cycle. Specifically, protein intake was suppressed during estrus for the presentation of the Fat-Protein and Protein-CHO diet pairs. These changes in protein intake with naturally-occurring fluctuations of the ovarian hormones lend creadence to the hypothesis that the failure to see a significant change (i.e., suppression) in protein intake during estrus with the diet triplet in Experiment 2 was due to the low baseline intake of this diet. Once elevated, not only was this suppression of protein seen during estrus but an increase in protein intake was seen during diestrus.

The data from these intact rats maintained with the Fat-Protein diet pair are incongruent for the caloric intake of fat in contrast to that seen with the diet triplet across the estrous cycle. Fat intake in this condition did not vary across the cycle. No reason currently exists for the lack of ovarian hormonal control over the intake of the fat diet when it is paired with protein. It should be noted, however, that total caloric intake does not vary across the estrous cycle for this diet pair, an effect similar to that for the Fat-CHO diet pair and the diet triplet. This suggests that, although not significant, a decrease in fat intake must occur during diestrus and a contrasting increase during estrus, given the changes observed for protein intake, however variability in the data must mask these effects on fat intake alone across the estrous cycle.

Consistent with the view that E2 suppresses the intake of CHO and protein is the finding that both are suppressed when presented together as the Protein-CHO diet pair during estrus and similarly enhanced during diestrus. The covariance of protein and CHO intake across the estrous cycle resulted in a decrease in body weight during estrus and increase during diestrus. This change in body weight across the estrous cycle is presumably due to the failure of the rats to select from a diet high in fat content.

Like that seen for the Protein-CHO diet pair, body weight also increased and decreased for the Fat-CHO and Fat-Protein diet pairs during diestrus and estrus, respectively. It therefore appears that all three basic macronutrient diet sources must be simultaneously present for the lack of change in body weight to occur. With the presence of the three diets, a more precise regulation of energy intake versus energy expenditure appears to occur.

Finally, no change in water intake was seen across the estrous cycle for any of the three diet pairs, a finding similar to that of the diet triplet.

CHAPTER VIII

EXPERIMENT 6: RESTRICTION OF DIETARY CHOICE AND THE ROLE OF EXPERIMENTALLY-ADMINISTERED ESTRADIOL ON MACRONUTRIENT SELF-SELECTION

The results of Experiment 5 demonstrated that, in general, the dietary self-selection patterns of the diet pairs could be predicted from the selection patterns of the diet triplet in Experiment 2. With the generality of the relations between naturally-occurring changes in estrogenic stimulation and diet selection established, the results of the relations found in the gonadectomy-replacement (E2) therapy paradigm using the diet triplet (i.e., Experiment 3) also seemed to warrant a test of further generality. Therefore, a group of ovariectomized rats was presented with the same diet pairs as in the prior experiment, injected with EB, and their dietary self-selection patterns observed.

Method

Subjects

Twelve adult female rats with a mean body weight of 284.5 ± 2.8 g received bilateral ovariectomies as described above.

Procedure

Two weeks post surgery, the rats had their maintenance diet of powdered chow (Purina Rodent Chow 5001) changed to the same equal

caloric diets and diet sequence as described in Experiment 5 (i.e., Fat-CHO, Fat-Protein, and Protein-CHO diet pairs).

Estrogen Replacement Therapy

One week after presentation of the Fat-CHO pair (three weeks postoperatively), each group was further divided into two groups matched on body weight and received EB injections according to the two injections sequences used in Experiment 1 (i.e., 3, 9 ug/kg body weight, oil or 9, 3 ug/kg body weight, oil). As in Experiment 1, a postinjection period occurred only its duration was 10 days in length, during which time the rats regained their body weights and pre-EB dietary self-selection patterns and intakes. Similarly, the next three days served as a baseline period for the next EB injection. After the second EB injection, an additional 10 days ensued, followed by a three day pre-oil injection period and a 10 day post-oil injection period. The same protocol was followed for the subsequent presentations of the Fat-Protein and Protein-CHO diet pairs. At the end of the presentation of the last diet pair the rats were killed.

Results

The caloric intake of each diet within each diet pair was analyzed with three, three-way ANOVAs (Macronutrient Diet X Dose X Time), one for each diet pair. The analysis of the caloric intake for the FAT-CHO diet revealed significant main effects of Macronutrient Diet, Dose, and the Macronutrient Diet X Dose interaction, F (1,10) = 18.22, $p < .005$, F (2,20) = 6.27, $p < .05$ and F (2,20) = 21.27, $p <$

.00001, respectively. Simple main effects tests were conducted for the Macronutrient Diet X Dose interaction resulting in significant effects of hormone treatment on consumption of both diets, CHO: F (1,33) = 8.21, $p < .01$ and Fat: F (1,33) = 5.23, $p < .05$. It was further found that the injections of both doses of EB enhanced the caloric intake of the fat diet (DNMR test, both $ps < .01$), however, there was no difference between the estrogenic stimulation of fat intake by the two doses of EB ($p > .1$). In contrast to the enhancement of fat intake by EB, CHO intake was suppressed by both EB doses (DNMR test, both $ps < .01$) and again, no difference was found between the effect of both doses of hormone ($p > .1$).

The caloric intake analysis for the Fat-Protein diet pair suggested significant main effects of Macronutrient Diet and Dose, F (1,10) = 21.3, $p < .001$ and F (2,20) = 4.03, $p < .05$, respectively. It was further found that the overall caloric intake of the protein diet was greater than that of the fat diet (DNMR test, $p < .01$) and that both protein and fat intake were suppressed by the two doses of EB (DNMR test, both $ps < .01$), but these doses did not produce a graded effect ($p > .1$).

Caloric intake of Protein-CHO intake resulted in a significant main effect of Dose, F (2,20) = 14.65, $p < .0001$. It was additionally found that both doses of EB suppressed the caloric intakes of CHO and protein (DNMR tests, both $ps < .01$) but did not differ with respect to one another ($p > .1$).

To summarize, EB replacement therapy suppressed the caloric intake of CHO and protein, regardless of the other diet in the diet pair. However, the effect of EB on fat intake was more complicated with enhancement seen when the other diet was CHO but suppressed if it was protein. These results are graphically depicted in the right half of Figure 7.2.

The effects of EB on total caloric intake were analyzed for each diet pair with a two-way ANOVA (Dose X Time). For all diets, there was a significant main effect of Dose, Fat-CHO: F (2,20) = 6.27, p < .005, Fat-Protein: F (2,20) = 4.03, p < .05 and Protein-CHO: F (2,20) = 14.65, p < .0001. Once again, both doses of EB suppressed total caloric intake for all diet pairs (DNMR tests, all ps < .05) but did not produce a graded estrogenic suppression (all ps > .1).

Daily water intake, as affected by EB injection, was analyzed with a four-way ANOVA (Hormonal Injection Sequence X Diet Pair X Dose X Time). This analysis found a significant effect of Diet Pair, F (2,20) = 6.69, p < .05. Water intake was subsequently found to be increased for any diet pair that had the protein diet as one of its members (DNMR test, p < .05).

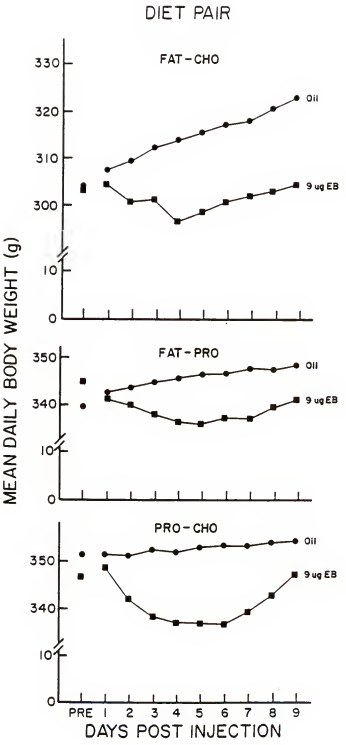
The effects of EB on body weight were analyzed with a four-way ANOVA (Hormonal Injection Sequence X Diet Pair X Dose X Time) and resulted in a significant main effect of Diet Pair, F (2,20) = 123.57, p < .00001 and Dose, F (2,20) = 68.10, p < .00001. Body weight of the rats when given the Fat-Protein or Protein-CHO diets was greater than when these rats were fed the Fat-CHO diet (DNMR test, both ps <

.01) but not different from each other ($p > .1$). The two doses of EB significantly suppressed the body weight of these rats, regardless of the diet pair (DNMR test, $p < .01$) but did not display a dose-response effect ($p > .1$). Figure 8.1 shows the effect of EB injection on body weight for the three diet pairs. Since the two doses of EB did not produce a differential suppression of body weight, only the 9 ug/kg dose of EB is shown in contrast with the oil vehicle for clarity.

Discussion

In all cases but one, the gonadectomy-replacement therapy with EB resulted in similar qualitative effects to that seen with the diet triplet in both intact, cycling rats during estrus (i.e., Experiment 2) and ovariectomized rats given EB injections (i.e., Experiment 4) for each diet of each diet pair. Only the intake of fat when paired with a protein diet differed from that of intact rats during estrus and ovariectomized rats given EB injections when presented with the diet triplet. In the present experiment, estrogenic stimulation resulted in a suppression of fat intake. For reasons unknown at this time, estrogenic stimulation does not result in enhanced fat intake if the fat diet is paired with a protein diet but does if it is paired with a CHO diet, as in this experiment or presented as part of a diet triplet (i.e., Experiments 2 and 3). As in the intact, cycling rats or ovariectomized rats given EB injections and presented with the diet triplet, estrogenic stimulation always suppressed the intake of either or both CHO or protein.

Figure 8.1: Mean body weight (g) for ovariectomized rats for the Fat-CHO, Fat-Protein, and Protein-CHO diet pairs after a s.c. injection of oil (circles) or 9 ug/kg EB (squares).


DIET PAIR
FAT-CHO
330
320
310
300
10
0
Oil
9 ug EB
FAT-PRO
350
340
10
0
Oil
9 ug EB
PRO-CHO
350
340
10
0
Oil
9 ug EB
MEAN DAILY BODY WEIGHT (g)
PRE 1 2 3 4 5 6 7 8 9
DAYS POST INJECTION

Contrary to the intact diet triplet and pair data, total kcal intake decreased after EB injection with no instance of "caloric compensation" occurring. That is, for the Fat-CHO and Fat-Protein diet pairs, injections of EB did not result in an increase in the caloric intake of the fat diet pair such that the suppression of CHO and protein were offset. Again, it would be naive to conceive of gonadectomy-replacement therapy with EB as producing the same physiological and behavioral effects as occur during estrus.

Body weight was found to decrease in rats with all diet pairs after EB injection, an effect seen during estrus for rats presented with the same diet pairs. As suggested for the intact diet pair body weight data, it may be that all three diets must be simultaneously available for caloric intake to match energy expenditure and therefore not alter body weight.

CHAPTER IX

GENERAL DISCUSSION

The results of these studies indicate that the ovarian hormones modulate dietary self-selection and, moreover, that this modulation appears to be primarily due to fluctuations in estrogenic stimulation. Estradiol, or its absence, appears to affect the selection of diet based on caloric density, with an inverse relation existing between estrogenic stimulation and the selection of diets high in caloric density, regardless of the major macronutrient in the diet. However, it should be noted that some macronutrient specificity exists in that the intake of CHO diets are more dramatically affected by E2. The general conceptualization based on the last forty years of research is that estrogen reduces food intake, a generalization that must be revised in light of the present findings. Specifically, E2 was, in general, shown to increase the intake of fat and decrease the intake of CHO, and, to a lesser extent, protein in both intact, cycling and ovariectomized rats receiving EB replacement therapy. Given these results, the previous observations that caloric intake increases during diestrus and after OVX, and decreases during estrus and after OVX with EB replacement therapy, are to be expected (for a review see Wade, 1974; Wade & Gray, 1979). That is, the nearly exclusive use of standard laboratory chow is, in realty, the use of a high

CHO (over 50%) and high protein (nearly 25%) diet (e.g., Purina 5001 Laboratory Rat Chow). Therefore, the typical changes in caloric intake seen across the estrous cycle, after OVX, and OVX plus EB replacement therapy can be accounted for by the relations between E2 and CHO and protein intake found in the present study.

Following OVX, it was observed during the development of OVX-induced obesity that the intake of CHO and protein progressively increased while that of fat decreased. It was also noted that these changes were most likely not due to a persisting, decremental influence of the ovarian hormones following the removal of their primary source since a similar pattern of intake was noted in ovariectomized rats first presented these diets one month after surgery. It therefore appears that the postingestive consequences of these diets influenced the probability of their future consumption. From the metabolic perspective exemplified by Friedman and Stricker (1976), it might be proposed that CHO and protein are utilizable metabolic fuels after OVX and that fat is not a viable metabolic fuel under these conditions. The reinforcing or aversive stimuli that occur postabsorptively are, of course, not known. These data, however, provide a focus for future research investigating whether these macronutrients are selected based on their utilizability as a metabolic fuel in contrast to olfactory and/or taste related factors. Regardless of the outcome of the future research, the use of the dietary self-selection paradigm has provided an orderly data base

from which to guide subsequent investigations, in contrast to experimenter generated tests of hypotheses often weak with repect to an underlying data base.

The relation between CHO intake and E2 has been recently extended to male rats as well (Siegel, Nunez, & Wade, 1981). The androgen testosterone can be converted to estradiol by 5 alpha-aromatase, an enzyme found in many peripheral tissues and brain (for review see Luttge, 1979). It has previously been found that the administration of high doses of testosterone propionate (TP) to castrated rats reduces food intake and body weight while the 5 alpha-reduced matabolite of TP, 5 alpha-dihydrotestosterone propionate (DHTP), which is nonaromatizable, does not decrease body weight, even at high doses (Gentry & Wade, 1976), when rats are fed standard laboratory rat chow (i.e., high CHO diet). Utilizing the dietary self-selection paradigm, Siegel et al. (1981) were able to demonstrate that the aromatizable androgen TP, at high doses, reduced CHO intake in castrated rats while the nonaromatizable androgen, DHTP did not. Thus, the decrease in CHO seen with high doses of TP appears to be mediated via the aromatization of testosterone to estrogenic metabolites. Therefore, these new data appear to extend the relation between E2 and CHO intake to male rats.

As was stated earlier (see General Introduction) this is not the first study to examine the role of the ovarian hormones in macronutrient self-selection. While the shortcomings of the previous studies were dealt with earlier, some of the findings of these studies that are not supported or are supported by the present work necessitate comment.

The recent work by Geiselman et al. (1981) was criticized for not using isocaloric diets. It should be recalled that these workers reported an apparent relation between CHO, fat, and E2 opposite to that seen in these studies. Because Experiment 1 clearly demonstrated that, in the absence of E2, a high caloric diet is consumed nearly exclusively regardless of its major macronutrient constituent, and that EB replacement therapy decreases the intake of these high caloric diets, the use of a pure fat diet that is 2.5 times more calorically dense than that of the CHO or protein diets would favor an increase in the consumption of fat during diestrus and after OVX with a decrease during estrus. These results were reported by Geiselman et al. (1981) in addition to an opposite dietary intake profile for CHO. It therefore appears that the relation between caloric density and estrogenic stimulation can readily account for these findings.

In spite of the apparent methodological faults with their experiments, Geiselman et al. (1981) report that protein intake is suppressed during estrus, but does not quite reach statistical significance, a finding very similar to the present study. However, the current set of experiments clearly shows that if the intake of protein can be elevated, both intact and ovariectomized rats suppress the intake of this macronutrient with estrogenic stimulation. It therefore appears that the difficulties involved in inducing the intake of protein by rats (e.g., Panksepp, 1978) and the consequent low basal intakes tend to obfuscate the effects of E2.

The macronutrient composition of complex diets was varied in the work by Wurtman and Baum (1980) in order to keep the diets isocaloric with respect to one another but differ in their percentage of protein. Yet, with the apparent difficulties associated with the interpretation of results using such a procedure, it was stated that protein intake is regulated during estrogenic stimulation. Moreover, in an attempt to examine protein intake regulation in OVX rats offered two isocaloric diets varying in protein (and CHO) content, results consistent with the present studies were actually found. Total protein intake in that experiment was not affected by EB administration and represented about 25% of the total caloric intake. In the present experiments, when the caloric intake of a protein diet represented a similar or lower percentage of total calories (i.e., Experiment 1, Low Protein diet and Experiment 2, Protein diet of the diet triplet), protein intake of these diets was not affected by exogenous or endogenous estrogenic stimulation. However, the macronutrient composition of the diets offered in the present studies only varied with respect to one macronutrient. Thus, the present findings support and extend those of Wurtman and Baum (1980) if the intake of protein does not represent more than 25% of the total calories consumed. If a larger percentage of total kcal consumed is as protein, estrogenic stimulation will suppress protein intake, as was found in the present study.

An additional finding by Wurtman and Baum (1980) was that estrogen decreased total caloric intake in intact, cycling and OVX rats

given EB injections. In both cases a separate diet source of fat was not made available. In the present set of studies, total caloric intake was most markedly affected when a separate fat source was not provided (i.e., Experiment 5, Protein-CHO diet--intact, cycling rats and Experiment 6, Protein-CHO diet--spayed rats given EB). Therefore, it seems possible that the decrease in total calories consumed, with estrogenic stimulation as reported by Wurtman and Baum (1980), may be due to the inability of the rats to increase their fat intake in response to elevated plasma levels of E2. In the present study, when a separate fat fource was available, total kcal intake was not changed by estrogenic stimulation (i.e., Experiment 1, diet triplet--intact, cycling rats; Experiment 5, diet pair: Fat-CHO & Fat-Protein--intact, cycling rats and Experiment 6, diet pair: Fat-CHO--spayed rats given EB) or nearly unchanged (Experiment 3, diet triplet--spayed rats given EB; however, for an exception see Experiment 6, diet pair: Fat-Protein--spayed rats given EB).

A major finding of the present investigation that has never been reported is that body weight does not change across the estrous cycle (i.e., Experiment 2, diet triplet). The change in body weight accompanying changes in the natural rhythms of the ovarian hormones across the estrous cycle, particularly E2, has been a topic of interest from the beginning of the study of these hormones in relation to energy balance (e.g., Brobeck et al., 1947) and the basis of four major reviews (Nance, in press; Wade, 1972, 1974; Wade & Gray, 1979). Typically, rats display a decrease and subsequent increase in body weight

during estrus and diestrus, respectively (e.g., Brobeck et al., 1947). However, it must be noted that these data were collected from rats eating standard laboratory chow as their sole source of calories. In the present study when three isocaloric diets, each high in content of one of the basic macronutrients, were offered, body weight did not change across the estrous cycle. In addition, total caloric intake did not change. While energy expended as locomotor energy or through thermoregulatory systems was not measured, it is tempting to speculate that the failure to see a change in body weight across the estrous cycle was primarily due to the apparent "regulation" of caloric intake and not to thermoregulatory adjustments (however, see Laudenslager et al., 1980) or locomotor activity changes. In regard to this last alternative, earlier work indicating that home cage motoric activities increase during estrus and decrease during diestrus (e.g., Finger, 1969), similar to that seen if cycling female rats are housed in activity sheels (e.g., Wang, 1923), has been seriously questioned in a recent study (Mullenix, 1981). It therefore appears safe to assume that a substantial proportion of the changes in body weight of intact, female rats observed across the estrous cycle reported previously (for review see Wade, 1972) was due to a single maintenance that was high in CHO content. Thus, these previous reports of weight change across the estrous simply reflects the result of the inverse relation between estrogenic stimulation and CHO intake reported in the present studies.

It is difficult to speculate about the physiological bases for the diet self-selection patterns seen in these experiments. This

difficulty in suggesting possible metabolic scenarios that may result in these diet selection patterns is due to the absence of any physiological measures correlated with the dietary selection profiles observed in any study examining the role of the ovarian hormones in dietary self-selection (e.g., Geiselman et al., 1981; Kanarek & Beck, 1980; Leshner & Collier, 1973; Richter et al., 1938, 1939; Wurtman & Baum, 1980). Thus, even the basic knowledge of the levels and/or functional changes in insulin secretion in intact, cycling and ovariectomized rats given EB injections is only known for rats consuming standard laboratory chow (Storlien, Martin, & Bellingham, 1979; Baily & Matty, 1972; Young, Schlussman, & Dixit, 1979) and not applicable here since diet influences insulin secretion (for review see Laube & Pfeiffer, 1978). Therefore, with the lack of these and other basic data, it is not possible to readily explain the relations between dietary self-selection and the ovarian hormones, particularly E2, in terms of changes in the availability of utilizable metabolic fuels by insulin or any other factor (Friedman & Stricker, 1976). Future research to be conducted by this investigator will examine such factors as glucose tolerance, brown adipose tissue thermogenesis, gluconeogenesis, ketogenesis and lipogenesis in intact cycling rats, rats developing OVX-induced obesity, and OVX-induced obese rats given EB replacement therapy. In addition, the estrogenic stimulation of tissues necessary and/or sufficient to produce the E2 diet selection patterns observed in this study will also be examined (e.g., central nervous system sites & peripheral tissues such as adipose, muscles, and pancreas).

Taken together with the testerone-EB suppression of CHO demonstrated in male rats (Siegel et al., 1981), the suggestion of an E2-induced suppression of CHO in intact cycling rats during estrus and in spayed rats administered EB (Wurtman & Baum, 1980), and the suppression of CHO intake during the ovulatory phase of the menstral cycle in rhesus monkeys (Kemnitz, Note 4), the present results strongly support and extend the suppression of the intake of CHO with estrogenic stimulation.

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BIOGRAPHICAL SKETCH

Timothy Jon Bartness was born in Albert Lea, Minnesota, on September 1, 1953. He graduated from Faribault Senior High School, Faribault, Minnesota, in 1971. His undergraduate work was conducted at Luther College, Decorah, Iowa, from 1971 to 1975, receiving his B.A. Degree with honors, majoring in biology, psychology, and history. He began his research career at Luther College with Drs. Werner Nitschke and William Moorcroft in the area of developmental psychobiology. From 1975 to 1977 he attended Drake University in Des Moines, Iowa, receiving his M.A. degree in general experimental psychology. During his tenure at Drake University, he conducted research in the area of the experimental analysis of behavior with Drs. Larry Alferink and William Klipec. In 1977, he began his doctoral work at the University of Florida, Gainesville, receiving his Ph.D. in psychology in 1982, majoring in psychology and specializing in physiological psychology. While at the University of Florida, he conducted studies in the area of ingestive behavior with Dr. Robert Waldbillig. During his last year, he was a research assistant in the laboratory of Dr. Neil Rowland where he continued his study of ingestive behavior.

He was married in 1974 to Sue Ellen Unger and has a daughter, Lacey Elizabeth, born in 1980. His future plans include a two year

postdoctoral tenure at the University of Massachusetts in Amherst, with Dr. George Wade, where he will continue his ingestive behavior research with special emphasis on their control by the gonadal hormones.

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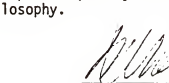

Merle E. Meyer, Chairman
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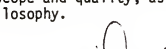

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Associate Professor of
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This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1982

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